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The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

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The *Journal* publishes original research articles and notes, critical reviews, Perspectives (essays of opinion or hypothesis), comments, and book reviews. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmatory state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

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Editorial

With this issue of the *Journal* we welcome four new Associate Editors and inaugurate a partially decentralized system for scientific editing of the *Journal*. Manuscript editing was previously a centralized function, handled solely in Ottawa. Now, only the Editor and Deputy Editor will be located in Ottawa. The latter will be responsible for the review and editing of about half of the manuscripts, and for coordinating the activities of the Associate Editors who have been delegated full authority for review and final disposition of the remaining submissions. This decentralized system is a variant of a group of systems used elsewhere for journal management, and we anticipate a smooth transition to its use.

All new manuscripts should be submitted to our headquarters office where they will be assigned to one of the scientific editors.

The 12 members of the Editorial Board will continue to assist the Branch in maintaining the stature and high scientific standards of the *Journal*. In their role as advisors, they will help to evaluate and formulate editorial policy, assess new submissions, arbitrate between antagonists in the case of contentious manuscripts, and encourage submission of the best papers in disciplines within the *Journal*'s scope.

One of the primary objectives of the newly reorganized Information and Publications Branch in DFO is to ensure that the *Journal* remains the leading outlet for the latest advances in fisheries and aquatic sciences that are relevant to Canadian interests. This requires that it attract and publish outstanding research results, maintain its internationally recognized standards of scientific excellence, and ensure that the information released is readily retrievable and widely disseminated. Given the continued support of our authors and reviewers, we intend to keep the *Journal* at the leading edge of fisheries and aquatic research.

As always, we welcome readers' comments and constructive criticism of our policies.

JOHANNA M. REINHART
Director and Editor

Éditorial

Avec le présent numéro du *Journal*, nous souhaitons la bienvenue à quatre nouveaux rédacteurs associés et nous inaugurons un système partiellement décentralisé de révision scientifique. La révision des manuscrits était auparavant centralisée, assurée seulement à Ottawa. Maintenant, il n'y aura à Ottawa que la rédactrice et le rédacteur adjoint. Ce dernier sera responsable de la révision et de la rédaction d'environ la moitié des manuscrits et de la coordination des activités des rédacteurs associés à qui on a délégué tout pouvoir pour la révision et l'utilisation finale des autres manuscrits soumis. Ce système décentralisé est une variante d'un groupe de systèmes utilisés ailleurs pour l'administration d'un journal et nous prévoyons que la transition vers son utilisation se fera sans heurts.

Tous les manuscrits doivent être soumis à notre bureau de l'administration centrale où on les attribuera à l'un des réviseurs scientifiques.

Les 12 membres du Conseil de rédaction continueront à aider la Direction à maintenir l'envergure et les normes scientifiques élevées du *Journal*. Dans leur rôle de conseillers, ils aideront à évaluer et à formuler la politique de rédaction, évalueront les nouveaux textes soumis, serviront d'arbitres entre les antagonistes dans le cas de manuscrits litigeux et encourageront la présentation des meilleurs documents dans des disciplines visées par le *Journal*.

Un des objectifs primordiaux de la Direction de l'information et des publications au MPO est de veiller à ce que le *Journal* demeure le principal organe de diffusion des plus récents progrès réalisés dans le domaine des sciences halieutiques et aquatiques et qui sont pertinents quant aux intérêts canadiens. Cela exige du *Journal* qu'il attire et publie des résultats de recherches marquantes, qu'il maintienne ses normes internationalement reconnues d'excellence dans le domaine scientifique et qu'il veille à ce que les informations divulguées puissent facilement être retrouvées et soient largement diffusées. Étant donné l'appui soutenu de nos auteurs et réviseurs, nous comptons garder le *Journal* à la fine pointe de la recherche halieutique et aquatique.

Comme d'habitude, les commentaires des lecteurs et une critique constructive de nos politiques sont bienvenus.

JOHANNA M. REINHART
Directrice et rédactrice

Trace Element Loadings to Sediments of Fourteen Ontario Lakes and Correlations with Concentrations in Fish

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Johnson, M. G. 1987. Trace element loadings to sediments of fourteen Ontario lakes and correlation with concentrations in fish. Can. J. Fish. Aquat. Sci. 44: 3–13.

Present, background, and anthropogenic loading rates of Cu, Ni, Zn, Pb, Cd, Cr, Hg, As, and Se to lake sediments were calculated from ^{210}Pb dated cores, and present loading rates were compared with concentrations in several fishes. Present loadings of Pb averaged 17 times background loadings and all lakes had statistically significant anthropogenic inputs. The majority of lakes had anthropogenic loadings of Zn, Cd, Hg, and As, which were presently 1.8–2.6 times background loadings. For Ni, Cu, Se, and Cr, only seven, five, two, and one lake(s), respectively, had significant anthropogenic inputs, and the means of present:background loadings for all lakes were not significantly different from 1.0. Enrichment by Zn, Cd, As, and especially Pb was greater closer to industrialized regions, while Hg enrichment was more widespread. Anthropogenic and precipitation loadings for Zn, Pb, Cd, and As were similar, suggesting that anthropogenic inputs are atmospheric and that current atmospheric loadings are mostly anthropogenic. [Hg] and [Cd] in fish were correlated with loadings to sediments, but [Cu] and [Zn] in fish were relatively constant within species, supporting the hypothesis that contaminants in fish would be correlated with inputs to sediments, while trace nutrients in ample supply would be unrelated to loading. [Cr] and [Se] in fish were intermediate in response to loading, but correlation between [Pb] in fish and loadings was not detected.

Les charges actuelles, naturelles et anthropiques de Cu, Ni, Zn, Pb, Cd, Cr, Hg, As et Se des sédiments lacustres ont été calculées à partir de carottes datées au ^{210}Pb, et les charges actuelles ont été comparées avec les teneurs observées chez plusieurs poissons. Les charges actuelles de Pb étaient en moyenne 17 fois supérieures aux charges naturelles et tous les lacs montraient un apport anthropique statistiquement significatif. Les eaux de la majorité des lacs présentaient des charges anthropiques de Zn, Cd, Hg et As, qui étaient à ce moment de 1,8 à 2,6 fois supérieures aux charges naturelles. Dans les cas du Ni, Cu, Se et Cr, seuls sept, cinq, deux et un lacs avaient respectivement des charges anthropiques significatives et les moyennes du ratio entre les charges actuelles et naturelles de tous les lacs n'étaient guère différentes de 1,0. L'enrichissement par le Zn, le Cd et le As, et plus particulièrement par le Pb, était plus marqué près des régions industrialisées, tandis que l'enrichissement par le Hg était plus répandu. Les charges anthropiques de Zn, Pb, Cd et As étaient semblables à celles des précipitations, ce qui laisse entendre qu'elles proviennent de polluants atmosphériques et que les charges atmosphériques actuelles sont en grande partie anthropiques. Le [Hg] et le [Cd] des poissons ont été corrélés aux charges des sédiments, mais le Cu et le Zn étaient relativement constants chez une même espèce. Ces données corroborent l'hypothèse selon laquelle les contaminants trouvés chez le poisson seraient corrélés avec les apports aux sédiments tandis que les éléments nutritifs très abondants en traces n'auraient aucun lien avec la charge. Le [Cr] et le [Se] chez le poisson étaient moyens par rapport à la charge, mais aucune corrélation entre le [Pb] chez le poisson et les charges n'a été établie.

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Metal concentrations in fish have been related to lake morphometry and pH (Wren and MacCrimmon 1983; Suns et al. 1980), alkalinity (Scheider et al. 1979), dissolved organic matter (Wiener and Geisy 1979), trophic relationships of fish (Rodgers and Qadri 1982), differences among species, and proportional to individual weight within populations (Wren et al. 1983). Measures of exposure to trace elements, such as ambient water and sediment concentrations and loadings, have been used rarely to interpret trace element concentrations in fish. Håkanson (1980) employed Hg content of surface sediments together with pH and an index of bioproduction to model Hg content of pike.

Measurements of mass sedimentation rates and trace element concentration profiles allow calculation of trace element loadings to sediments. These should reflect inputs to lakes and serve as an index of exposure to trace elements by fish. Temporal and spatial trends in trace element loadings have been determined from analysis of sediment core profiles in many areas (Kemp and Thomas 1976; Kemp et al. 1978; Galloway and Likens 1979; Evans and Dillon 1982; Johnson et al. 1986). Increased trace element loadings to lakes were attributed usually to anthropogenic sources including loadings from the atmosphere to undeveloped lakes. To date, loadings of trace elements have not been compared with concentrations in fish.

The main objective of this study was to compare loadings of trace elements (Cu, Ni, Zn, Pb, Cd, Cr, Hg, As, and Se) with concentrations in several fish species in a widespread sample of Ontario lakes. Contaminants (not nutritionally significant) such

FIG. 1. Location of 14 lakes from which sediment and fish samples were taken. Boundary of the Precambrian Shield is shown.

as Pb, Cd, and Hg in fish were expected to be related to trace element loading rates. Micronutrients such as Cu and Zn were expected to show more uniform concentrations in fish among lakes. A second objective was to determine the magnitude of and geographic patterns in anthropogenic loadings (and present relative to background loadings) of the nine trace elements and to compare these with precipitation loadings.

Methods

Selection of Lakes and Fish Species

A total of 14 lakes, six pairs of closely located and two larger single lakes, were selected by type (salmonid or percid lakes), location (from far northwestern to southeastern Ontario), and logistic considerations (Fig. 1). Almost all lakes are small, except Manitou and Opeongo, a large hardwater and a large softwater lake, respectively (Table 1). Each of the two main basins of Manitou and three basins of Opeongo were sampled. The lakes are not acidified, eutrophied, or subject to problems associated with pollution by point-sources or runoff. They were selected as typical lakes in their respective geological settings. Only two lakes (Savanne and Henderson) have coloured waters but these lack the low pH of dystrophic lakes. Two others (Emmett and Bartley) have marl deposits in shallow coves. The four northwestern Ontario lakes occur in granitic basins and have soft waters, with alkalinities of 6–15 $mg \cdot L^{-1}$. The central group of lakes (Manitou, Sucker, Windfall, Emmett, and Bartley) are located on Lower Silurian dolostone formations and have alkalinities of 105–152 $mg \cdot L^{-1}$. The southeastern group includes Opeongo, Barker, Ashby, Antoine, and Ardoch, all of which are located on the Precambrian Shield. The latter two have relatively high alkalinity (Table 1) because carbonate metasediments occur in their basins. Barker and Ashby lakes occur in granitic basins, and the basin of Opeongo is partly granitic and partly clastic metasediments. These three lakes have alkalinities of 10–14 $mg \cdot L^{-1}$.

The fishes selected were ubiquitous species to allow comparisons across all lakes sampled (common white sucker, *Catostomus commersoni*, and yellow perch, *Perca flavescens*) and species of greater interest in recreational fisheries (lake trout, *Salvelinus namaycush*, and lake whitefish, *Coregonus clupeaformis*, from the softwater lakes and walleye, *Stizostedion vitreum*, and northern pike, *Esox lucius*, from the hardwater lakes). This variety of species also provided information on variability in trace element concentrations attributable to species. An average of 11 specimens per species were collected in each lake, and a total of 426 individual fish were analysed.

Collection and Analysis of Sediments

Because mass sedimentation and trace element loading rates are usually at a maximum in the main depositional basin of lakes (Dillon and Evans 1982), core samples were taken consistently from the deepest point of each lake (or basin in the case of Manitou and Opeongo). Sediment cores were collected in 1977–80 utilizing a KB corer modified to accept Benthos corer tubes of 6.6-cm diameter. Cores were sliced horizontally at 1-cm intervals to 10 cm, at 2-cm intervals to 20 cm, and at 5-cm intervals in the remainder. The extruder discarded the outer 0.8 cm to reduce effects of smearing along the inner surface of the core tube.

Subsamples were freeze-dried and subsequently digested in nitric and hydrochloric acids and analysed for Cu, Ni, Zn, Pb, and Cd utilizing a Varion AA-6 atomic absorption spectrophotometer by Bondar-Clegg Ltd. of Ottawa, Ont. Other subsamples were digested in nitric and perchloric acids and As and Se concentrations were determined by colorimetric techniques on a Bausch and Lomb Spectronic 700 spectrophotometer. Mercury was determined using cold vapour generation techniques. Detection limits for these trace elements were as follows: Cu, Ni, Pb, Cr, and As, 0.5 $\mu g \cdot g^{-1}$; Se, 0.25 $\mu g \cdot g^{-1}$; Cd and Zn, 0.1 $\mu g \cdot g^{-1}$; Hg, 5 $ng \cdot g^{-1}$. Precision on replicate samples was good for Cu, Ni, Zn, Pb, Cd, and Cr (3–10% coefficient of variation, $n = 10$) and fair for Hg, As, and Se (15–30% coefficient of variation, $n = 10$).

Sedimentation Rates

A single core from each set was sectioned, slices were dated, and mass sedimentation rates were calculated, using ^{210}Pb and ^{137}Cs methods (Durham and Joshi 1984). Flett Research Ltd. of Winnipeg, Man., analysed cores from the two ELA lakes. R. W. Durham and S. R. Joshi at the National Water Research Institute in Burlington, Ont., determined mass sedimentation rates in the rest of the cores. Uniform sedimentation through time was indicated by linear relationships between $\log_e$ ^{210}Pb activity and cumulative sediment mass with depth (Durham and Joshi 1984).

Analyses of Fish Samples

Individual whole fish were homogenized, freeze-dried, and submitted to Bondar-Clegg Limited for the same trace element analysis performed on the sediments. Percent moisture was determined so that elemental concentrations could be expressed on a wet weight basis. Reference samples of known trace element concentrations were submitted with the other samples. Results were within 20% of reference values of Cu, Ni, Hg, and Se. Precision was variable, 9% (coefficient of variation, $n = 12$) for Cu, 16% for Zn, and close to 50% for Cr and Se. The majority of Ni, Pb, and As concentrations in fish were less

TABLE 1. Locations, morphometry, pH, alkalinity of 14 lakes examined in this study.

Lake	Location	Area (ha)	Depth		pH	Alkalinity (mg $CaCO_3 \cdot L^{-1}$)
			Max. (m)	Mean (m)		
Northwestern group						
ELA 305	49°41′N, 93°40′W	52	32.7	15.1	7.1	6
ELA 468	49°40′N, 93°45′W	290	36.0	—	7.1	6
Savanne	48°50′N, 90°06′W	364	4.3	2.6	7.1	15
Henderson	48°49′N, 90°18′W	151	5.3	2.4	7.2	15
Central group						
Windfall	45°42′N, 82°05′W	466	15.3	3.2	8.8	134
Sucker	45°43′N, 81°52′W	233	7.6	2.2	8.0	105
Emmett	45°13′N, 81°28′W	99	10.6	2.4	7.6	129
Bartley	45°13′N, 81°29′W	33	3.6	1.5	6.7	152
Manitou	45°47′N, 82°04′W	10 311	50.0	16.0	8.2	112
Southeastern group						
Ashby	45°05′N, 77°21′W	260	36.6	4.3	7.0	14
Barker	45°07′N, 77°23′W	141	38.1	4.1	6.5	14
Antoine	45°00′N, 76°44′W	82	12.5	3.1	8.0	68
Ardoch	44°56′N, 76°52′W	96	17.4	7.6	7.5	154
Opeongo	45°42′N, 78°22′W	5 860	51.8	14.8	6.8	10

than detection levels. A second set of samples was prepared and submitted to Barringer-Magenta Ltd. of Toronto, Ont., to be analysed for Pb with a detection limit of 10 $ng \cdot g^{-1}$. Two standard reference samples (National Bureau Of Standards oyster and bovine liver tissues) were analysed and results were within 10% of known values and precision was 7% (coefficient of variation, $n = 5$) for oyster tissue. Budget constraints prevented the resubmission of samples for more accurate determinations of Ni and As. Since concentrations of Ni, As, and occasionally Cd were less than detection limits, precise mean concentrations could not be determined. In these cases, ranges were calculated by considering values below detection levels to be zero at minimum and at the detection level at maximum. Upper and lower values were sufficiently close to be useful.

Precipitation Loadings

Loadings in precipitation were obtained from several sources. Cu, Ni, Zn, Pb, and Cd loadings for 1981 were from "wet-only" collectors of the Ontario Ministry of the Environment (1983). Four stations closest to each lake group (northwestern, central, and southeastern) were used. The northwestern group consisted of ELA, Ear Falls, Pickle Lake, and Dorion, the central group included Killarney, Shallow Lake, Coldwater, and McKellar, and the southeastern group included Dorset, Kaladar, Wilberforce, and Whitney (Ontario Ministry of the Environment 1983, table 13 and fig. 1A). Precipitation loadings of As and Se were obtained from Traversy et al. (1975) but only their data from rural, and not urban, sites were included. Cr data were from Kramer (1976). The Hg loading was based on 65 bulk samples collected in 1980 and 1981 at Emmett Lake near Tobermory, Ont., in paired 30.5-cm Nalgene funnel collectors. Every rainfall event and the snowpack were sampled. Approximately 50% of sample results were above detection level. Lower and upper estimates of mean loading were obtained by considering concentrations below detection level as zero or at detection level, respectively, and by using Environment Canada daily precipitation records at Wiarton.

TABLE 2. Mass and linear sedimentation rates to main basins of the study lakes. Three basins in Lake Opeongo and two basins in Lake Manitou were sampled.

Lake	Mass sedimentation ($mg \cdot cm^{-2} \cdot yr^{-1}$)	Linear sedimentation ($mm \cdot yr^{-1}$)
Northwestern group		
ELA 305	14.3	1.34
ELA 468	18.0	2.07
Savanne	6.2	0.49
Henderson	17.1	1.48
Central group		
Windfall	4.6	1.26
Sucker	6.5	1.65
Emmett	2.6	0.54
Bartley	3.9	1.08
Manitou	9.7–11.6	1.05
Southeastern group		
Ashby	14.8	1.05
Barker	16.9	1.17
Antoine	5.6	1.28
Ardoch	5.3	1.04
Opeongo	8.3–12.3	0.79–1.55

Results

Sedimentation Rates

Mass sedimentation rates in main basins of the lakes varied from 2.6 to 18 $mg \cdot cm^{-2} \cdot yr^{-1}$ and averaged 9.8 with a standard error of 1.5 $mg \cdot cm^{-2} \cdot yr^{-1}$. Linear deposition rates averaged 1.2 $mm \cdot yr^{-1}$ with a standard error of 0.11 $mm \cdot yr^{-1}$ (Table 2).

Mass sedimentation rates were significantly greater in softwater Precambrian Shield lakes than in hardwater lakes, 14.0 compared with 5.6 $mg \cdot cm^{-2} \cdot yr^{-1}$. This variation may be related to differences in morphometry (that is, the size of sedimentation zones relative to lake size and the extent of concentration of sediment by area) or to differences in input, or both.

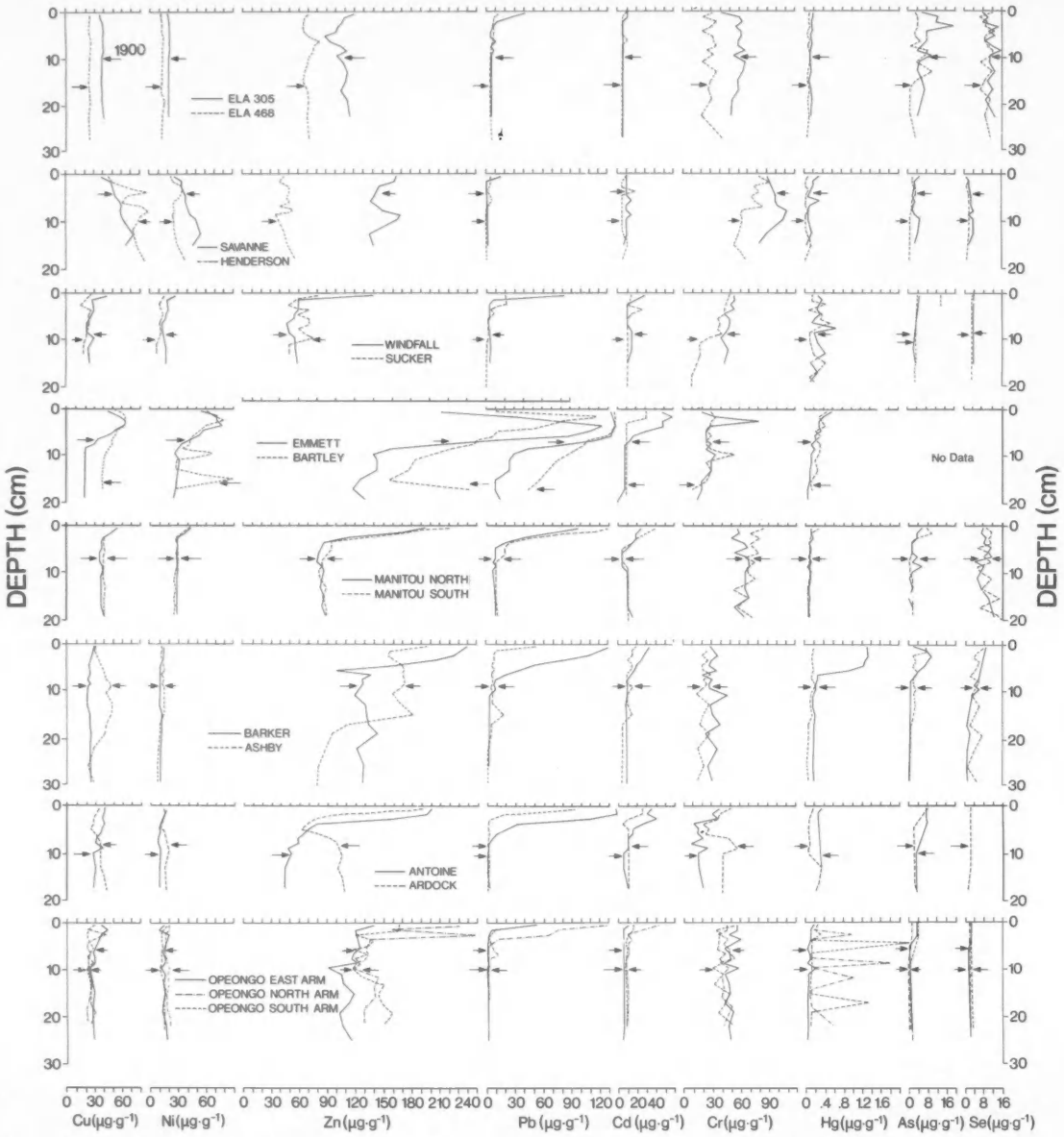

FIG. 2. Concentration profiles in sediments of nine trace elements in the 14 lakes sampled. Arrows indicate depths corresponding to the year 1900. Mean background concentrations were obtained from pre-1900 concentrations. Lakes are arranged vertically in west–east order approximately.

Trace Element Concentrations

Surface concentrations in cores generally exceeded background concentrations but the average difference varied among trace elements from 6% to close to 900% (Fig. 2). Cu, Ni, Cr, and Se had marginal differences, 6–25% over background. Zn, Cd, Hg, and As surface concentrations were 60, 167, 134, and 114% greater, respectively, than background. The surface concentration of Pb was 900% of background on average. Further interpretation of vertical profiles of trace elements will be made subsequently in terms of loadings.

The order of abundance of trace elements in background sediments was Zn > Cr > Cu > Ni > Pb > Se > As > Cd > Hg. In surface sediments (0–1 cm), Pb concentration was greater than all trace elements except Zn. Surface concentrations of Cu and Ni were significantly correlated ($P < 0.01$). Surface concentrations of Zn, Pb, and Cd were all highly significantly correlated ($P < 0.01$), while Hg concentrations were

TABLE 3. Present loadings (P) and background loadings (B) ($\mu g \cdot cm^{-2} \cdot yr^{-1}$) of trace elements to main basin lake sediments. Asterisks indicate the present loading rates based on concentrations at 0–1 cm significantly greater than background concentrations.

Lake (basin)	Cu	Ni	Zn	Pb	Cd	Cr	Hg	As	Se
Northwestern group									
ELA305									
P	0.52	0.27	1.73*	0.60*	0.014*	0.59	0.0028*	0.086	0.114
B	0.54	0.28	1.57	0.09	0.007	0.83	0.0018	0.076	0.162
ELA468									
P	0.43	0.22	1.28	0.25*	0.018*	0.58	0.0020*	0.054	0.216
B	0.43	0.24	1.27	0.09	0.009	0.51	0.0016	0.039	0.180
Savanne									
P	0.24	0.16	1.02	0.10*	0.006	0.56	0.0020*	0.030	0.010
B	0.38	0.28	0.92	0.01	0.006	0.62	0.0008	0.018	0.018
Henderson									
P	0.79	0.39	0.75	0.09*	0.017	1.40	0.0016*	0.030	0.020
B	1.36	0.54	0.86	0.02	0.011	1.20	0.0006	0.020	0.020
Central group									
Windfall									
P	0.20*	0.13*	0.64*	0.38*	0.014*	0.25	0.0014	0.023*	0.013
B	0.12	0.08	0.26	0.02	0.006	0.20	0.0011	0.012	0.018
Sucker									
P	0.17	0.10	0.53*	0.14*	0.010*	0.32	0.0007*	0.023*	0.018
B	0.15	0.07	0.28	<0.01	0.006	0.23	0.0002	0.012	0.018
Emmett									
P	0.11*	0.14*	0.54*	0.45*	0.012*	0.09	0.0015*	—	—
B	0.05	0.08	0.35	0.08	<0.002	0.08	0.0005	—	—
Bartley									
P	0.22*	0.24*	1.56*	0.80*	0.012*	0.12	0.0019*	—	—
B	0.15	0.11	0.95	0.18	<0.004	0.12	0.0006	—	—
Manitou (north)									
P	0.64*	0.50*	2.27*	1.14*	0.029*	0.67	0.0016	0.070*	0.104
B	0.44	0.34	0.99	0.11	0.011	0.76	0.0011	0.026	0.105
Manitou (south)									
P	0.59*	0.42*	2.16*	1.24*	0.039*	0.83	0.0028*	0.077*	0.097
B	0.40	0.26	0.83	0.11	0.010	0.66	0.0009	0.009	0.097
Southeastern group									
Ashby									
P	0.44	0.18	2.89	0.74*	0.030*	0.28	0.0030*	0.100*	0.120*
B	0.57	0.17	1.86	0.07	0.012	0.30	0.0019	0.024	0.058
Barker									
P	0.49*	0.25*	4.06*	2.47*	0.057*	0.49	0.0052*	0.033*	0.152*
B	0.40	0.19	2.13	0.07	0.017	0.55	0.0033	0.015	0.036
Antoine									
P	0.22	0.08*	1.12*	0.77*	0.019*	0.21*	0.0020	0.043*	0.016
B	0.17	0.05	0.25	<0.01	0.005	0.10	0.0018	0.011	—
Ardoch									
P	0.18	0.09	1.01*	0.42*	0.013*	0.26	0.0009	0.042*	0.013
B	0.20	0.08	0.55	<0.01	0.005	0.23	0.0008	0.016	0.012
Opeongo (east)									
P	0.24	0.17*	1.02*	0.45*	0.010	0.34	0.0014*	0.028*	0.015
B	0.23	0.12	0.69	<0.05	<0.007	0.31	0.0004	0.004	0.010
Opeongo (north)									
P	0.33	0.26	1.75*	0.74*	<0.01	0.24	0.0029*	0.019*	0.021
B	0.30	0.25	1.31	<0.06	<0.01	0.24	0.0011	0.006	0.015
Opeongo (south									
P	0.31	0.18	1.42*	0.53*	0.025*	0.36	0.0024*	0.042*	0.020
B	0.27	0.17	1.12	0.05	<0.009	0.35	0.0012	<0.005	0.015

significantly correlated with those of Zn and Pb ($P < 0.05$). Differences in background concentrations between softwater and hardwater lakes were small (≤26%) for all trace elements except Pb. Background Pb concentrations in hardwater lakes averaged about 3 times those in softwater lakes although the difference was not statistically significant.

Trace Element Loadings

In general, absolute values of loadings are of less relevance (because of within-lake variability) than the ratio of present to background loadings and confirmation of the presence of anthropogenic loadings by statistical tests on concentration pro-

files. Present loadings were considered to have a statistically confirmed anthropogenic component when the trace element concentrations in core slices exceeded the mean background concentration plus two standard deviations. The anthropogenic loading is the difference between present and background loadings.

The nine trace elements are categorized in three groups based on two criteria, the proportion of lakes which had significant anthropogenic inputs and present:background trace element loadings (Table 3).

Group 1: Pb

The first group (Pb only) had present:background loadings of 3–77 and they averaged 17. All cores showed significant surface enrichment. Therefore, the ratio of anthropogenic to background loadings was 16, that is 17 minus 1. Present and background loadings averaged 0.67 and 0.06 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively. Eight of the nine northwestern and central lakes had lower than average present:background loadings (4.4–14.0), and four of the five lakes in southeastern Ontario had greater than average present:background loadings (17.8–77.0).

Group 2: Zn, Cd, Hg, and As

Trace elements in the second group had significant anthropogenic inputs in the majority of lakes sampled and present:background loadings ranged from approximately 2 to 3. The average ratio for Zn was 1.8, and significant anthropogenic inputs occurred in 10 of the 14 lakes. Cd and Hg had present:background loadings averaging 2.6 and 2.2 and had significant anthropogenic inputs in all but two and three lakes, respectively. In the case of As, 7 of 12 lakes (no data on As for Emmett and Bartley) had significant anthropogenic inputs and present:background loadings averaged 2.7.

Present and background loadings of Zn averaged 1.51 and 0.95 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively, in all cores. The highest present:background loadings (>1.5) occurred in all lakes of the central and southeastern groups, and all but 1 (Ashby) of 11 lakes had detectable anthropogenic loadings. All northwestern lakes had ratios <1.2 and three of the four lakes did not have significant anthropogenic loadings.

Present loadings of Cd averaged 0.020 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, while background loading rates averaged 0.008 $\mu g \cdot cm^{-2} \cdot yr^{-1}$. Savanne and Henderson in northwestern Ontario were the only lakes that did not have significant anthropogenic inputs of Cd. Present:background loadings were >2.5 in most central and southeastern lakes (except Sucker Lake), while the ratio in northwestern lakes did not exceed 2.0.

Present and background loadings of As averaged 0.046 and 0.020 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively. Present:background loadings were >1.9 in central and southeastern lakes in which all anthropogenic loadings were significant. The four lakes that did not have statistically significant anthropogenic inputs of As were those in northwestern Ontario and they had present:background ratios of 1.1–1.6.

Present and background loadings of Hg averaged 0.0021 and 0.0012 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively. Lakes in the northwestern group had present:background loadings as high as the general average, and all four lakes had significant anthropogenic inputs. The three lakes without detectable anthropogenic inputs were lakes with higher alkalinity. Antoine and Ardoch lakes had the two lowest ratios and present and background loadings were not statistically different, as occurred also in Windfall Lake.

Group 3: Cu, Ni, Cr, and Se

The third trace element group had a minority of lakes with detectable anthropogenic inputs and the average present:background ratio was not significantly different from 1. This group included Ni but it was borderline with 7 of 14 lakes showing significant anthropogenic inputs.

The mean present Cu input was the same as average background input, 0.36 $\mu g \cdot cm^{-2} \cdot yr^{-1}$. Four of the five lakes with significant anthropogenic loadings were in the central group of lakes. Savanne and Henderson actually had significantly less present loadings to sediments than background loadings.

Present Ni loadings averaged 0.22 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, while background loadings averaged 0.19 $\mu g \cdot cm^{-2} \cdot yr^{-1}$. Both basins of Lake Manitou had higher than average present loadings and these were significantly different than background loadings. Emmett, Bartley, and Windfall had significant anthropogenic inputs although the present loading rates were not much higher than background rates. These four lakes are in the central group. Three lakes of the southeastern group, Barker, Antoine, and Opeongo (East Arm only), also had significant anthropogenic loadings.

There was a single core, from Antoine Lake, which had a significantly greater present loading rate of Cr than its background loading. Otherwise, present and background loadings of Cr were similar, averaging 0.45 and 0.43 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively, for all cores.

Only two lakes, Barker and Ashby, had anthropogenic Se inputs. Present loadings were 4.2 and 2.1 times background rates in the two lakes. Average present and background loadings were 0.063 and 0.055 $\mu g \cdot cm^{-2} \cdot yr^{-1}$, respectively, for the 12 lakes with Se data. Four softwater lakes (ELA 305, ELA 468, Barker, and Ashby) had approximately 10 times higher Se loadings than all hardwater lakes except Manitou. Lake Opeongo, also a softwater lake, had similar Se loadings to hardwater lakes. The two coloured-water lakes, Savanne and Henderson, had relatively low Se loadings.

Temporal Trends

The approximate time of first appearance of anthropogenic inputs was taken at the depth at which the loading rate first exceeded the upper confidence limit on the background loading rate. This method, although it was objective, produced quite variable results. The earliest anthropogenic loadings of Pb occurred in the period 1919–49 (mean ± 2× standard error). Corresponding times were 1926–66 for Zn, 1938–62 for Cd, and 1921–57 for Hg. Anthropogenic inputs (Pb and Hg mainly) detected in northwestern Ontario lakes were more recent on average, up to only 24 yr before present, than in most of the other lakes. Intensive work on Found Lake, Ont., by Evans and Dillon (1982) showed earliest anthropogenic Pb loadings by about 1900. Similarly, anthropogenic inputs of Pb, Zn, Cd, and Hg were detected as early as 1900–05 in sediment cores from five lakes of the Turkey Lakes system in Ontario (Johnson et al. 1986).

Trace Element Concentrations in Fish

Correlation of fish weight and trace element concentrations in fish was examined to assess the need to adjust concentrations to standard weight before examining the relationship between concentrations in fish and loadings. In the case of Hg, 23 of 40 series had significant positive relationships, while almost all of the other 17 series were limited by number of specimens and/or

TABLE 4. Mean concentrations (μg wet wt·g^{-1}) of trace elements in fish of six species taken from the study lakes. Where ranges are given the calculations are explained in the text. The mean concentration for each species in each lake was adjusted to a common reference weight when a statistically significant relationship was observed between trace element concentration and weight of individuals. Reference weights were 500 g for lake trout, whitefish, and walleye, 1000 g for common sucker and northern pike, and 100 g for yellow perch.

Species	Cu	Ni	Zn	Pb	Cd	Cr	Hg	As	Se
Lake trout	0.93	0.2–0.30	14.1	0.17	0.04–0.05	0.23	0.18	0.06–0.09	0.78
Whitefish	0.77	0.3	16.2	0.26	0.04–0.05	0.23	0.10	0.07–0.09	0.84
Common sucker	1.17	0.2–0.3	16.6	0.16	0.04–0.05	0.27	0.09	0.04–0.8	0.55
Yellow perch	0.90	0.2–0.3	19.5	0.19	0.04–0.05	0.21	0.10	<0.05	0.38
Northern pike	0.87	0.5–0.6	36.9	0.12	<0.02	0.19	0.20	<0.05	0.37
Walleye	0.60	<0.1	14.4	0.05	<0.02	0.22	0.13	<0.05	0.25

TABLE 5. Results of correlation analysis of trace element concentrations in fish (data from all species pooled) and trace element loadings. Values in parentheses are standard errors of the means. Double asterisks indicate significance at $P < 0.01$. Data on Ni and As in fish were inadequate for this analysis.

Trace element	Mean concentration in fish (μg wet wt·g^{-1})	Mean loading rate (μg·cm^{-2}·yr^{-1})	Metal concentration in fish as function of loading rates	Value of r
Cu	0.92 (0.052)	0.37 (0.031)	Cu = 1.030 − 0.284 Cu LR	−0.169
Zn	16.38 (0.636)	1.56 (0.153)	Zn = 14.63 + 1.116 Zn LR	+0.269
Pb	0.16 (0.015)	0.62 (0.098)	Pb = 0.177 − 0.023 Pb LR	−0.159
Cd	0.034 (0.007)	0.02 (0.002)	Cd = −0.0130 + 2.320 Cd LR	+0.742**
Cr	0.23 (0.016)	0.51 (0.055)	Cr = 0.149 + 0.160 Cr LR	+0.584**
Hg	0.087 (0.009)	0.0021 (0.171)	Hg = 0.0033 + 39.34 Hg LR	+0.728**
Se	0.528 (0.056)	0.069 (0.011)	Se = 0.354 + 2.52 Se LR	+0.494**

range in weight. In all species except whitefish, Hg residues increased with increasing weight. In decreasing order of occurrence, significant correlations between weight and trace element concentration were observed in only 5 of 40 possible cases for Zn, 3 each for Cu and Ni, 2 for Cr, 1 each for Se and Cd, and in no case for Pb and Cr. Data for testing weight − trace element correlations for Ni and As were inadequate because detection levels were often too high. Therefore, only Hg concentration in fish was generally correlated with body weight. Where regressions were significant, concentrations of trace elements were adjusted to standard weights of 1000 g for common sucker and northern pike, 500 g for lake trout, whitefish, and walleye, and 100 g for perch, and these values were used in further analysis.

Generally, variations among species in trace element concentrations were not great, with a few exceptions. One notable difference among fish species was significantly higher Zn concentrations in pike than in other species (Table 4). This trend was not related to higher Zn concentrations in sediments of lakes where pike were collected. Hg concentrations were higher in lake trout, northern pike, and walleye than in other species, possibly attributable to food-chain biomagnification. Higher Se levels in lake trout and whitefish might have been related to higher Se concentrations in sediments of the lakes from which these species were collected.

Concentrations of trace elements in fish occurred in the order Zn > Cu > Se > Ni > Cr > Pb > Hg > As > Cd (Table 4). Compared with a ranking of trace element sediment concentrations and loadings, Se and Hg were relatively higher in rank in fish than in sediment and Pb and Cr were relatively lower. If loading to lake sediments is indicative of relative exposure of fish to this series of trace elements, then the ratio of trace element concentration in fish to trace element loading rate should reflect the potential for net uptake. This ratio was highest at 69 in the case of Hg and lowest for Pb at 0.1. Zn, Se, and Cu had ratios of 15.5, 12.0, and 2.4, respectively. The ratio was close to 1.0 for Ni, Cd, and As, while Cr had an average ratio of 0.4. This measure of relative potential for net uptake by fish ranked the trace elements in the order Hg > Zn > Se > Cu > Cd > As > Ni > Cr > Pb.

Correlation of trace element concentrations in fish and loadings was examined, first with each species separately and then with all 40 species–lake combinations. To effectively remove differences that were significant and attributable to species (in the case of four species − trace element combinations), concentrations were adjusted so that the means were located on the line for the other species. Hg concentrations in lake trout, pike, and walleye and Zn concentrations in pike were adjusted by this method. Few significant regressions were obtained for species tested individually, in part because of small sample size. Concentrations of Cd and Hg in common white sucker and yellow perch and concentrations of Cr in lake trout and whitefish were positively related to loadings. When data from all species were pooled, correlations between tissue concentrations and trace element loading rates were highly significant in the cases of Cd, Cr, Hg, and Se (Table 5). No significant correlations were found in the cases of Cu, Zn, and Pb. Data on Ni and As concentrations in fish were inadequate for this analysis. Lake alkalinity, when it was included as a second independent variable in multiple regression, showed no significant relationship with trace element concentrations in fish. Slopes for Cu, Zn, and Pb were not significantly different from zero and variances were relatively low, which suggested approximately constant uptake and little effect by differences in loadings (Table 5). In contrast, Cd and Hg showed the reverse, where slopes were significant and intercepts were not significantly different from

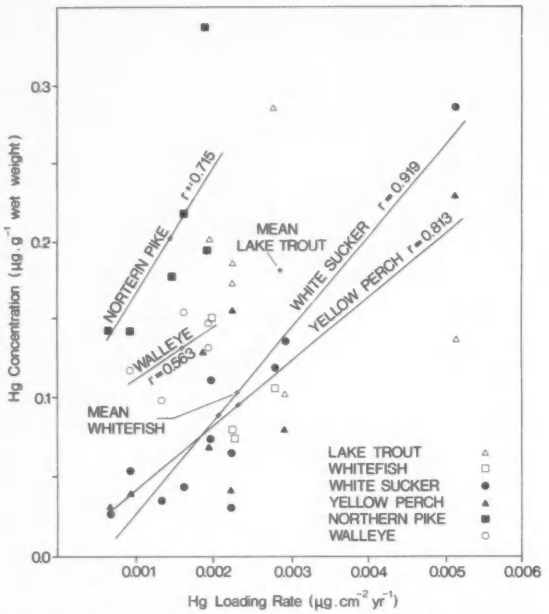

FIG. 3. Relationships between Hg loading rate and Hg concentrations in six species of fish.

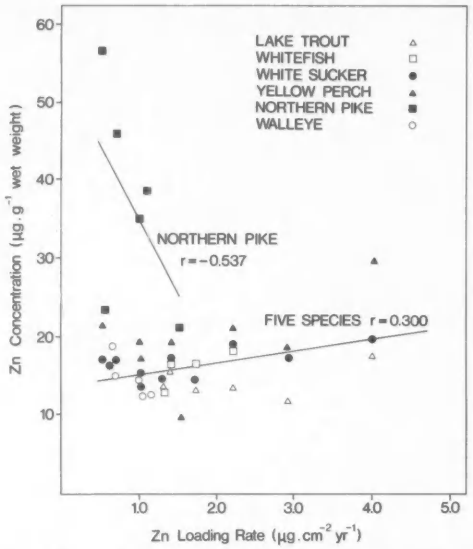

FIG. 4. Relationships between Zn loading rate and Zn concentrations in six species of fish.

zero. Therefore, loading rate largely determined concentration of Cd and Hg in fish. In the cases of Cr and Se, intercepts were substantial (significantly different from zero) and significant slopes showed that loading rates also were important in determining concentrations in fish.

The relationships between concentrations and loadings for Hg (a contaminant) and Zn (a micronutrient) are compared further. Comparison of concentrations of Hg among species as functions of Hg loading to sediments (Fig. 3) indicated that white sucker and yellow perch had similar means and slopes and whitefish had similar mean Hg concentration. Combined data for these three species produced a highly significant regression, in which 71% of the observed variation was accounted for by loading rate. When the higher means for piscivores were adjusted to eliminate species effects and combined with all other data, loadings accounted for 53% of the variation observed in Hg concentrations in fish. In contrast, the concentrations of Zn in fish appeared to be independent of Zn loading (Fig. 4). Higher concentrations in pike were peculiar to this species and not associated with higher loadings.

Discussion

Regional and Geological Patterns in Sedimentary Trace Elements

The regional pattern observed in present:background loadings and the strong correlation among surface concentrations of Pb, Zn, and Cd indicated that sediment Zn, Cd, and especially Pb are increasingly dominated by anthropogenic inputs in closer proximity to industrialized regions. The four lowest values of present:background loadings of Zn were in the four northwestern Ontario lakes, and present:background loadings of Pb and Cd were much lower than average. The few lakes that did not show significant anthropogenic inputs of Zn and Cd were mainly in northwestern Ontario. Only the four northwestern lakes did not have statistically significant anthropogenic inputs of As.

Hg also showed significant anthropogenic inputs in a large majority of lakes but these were more extensively distributed including some high anthropogenic loadings in northwestern Ontario.

Concentrations of Cu and Ni were correlated and almost all of the lakes that showed anthropogenic inputs of Cu and Ni were within 100 km of Sudbury. Crocket and Kabir (1981) showed enrichment of recent sediments by Cu and Ni in cores from lakes in the Sudbury–Temagami area attributable to smelting operations.

There were no regional patterns in Cr and Se concentrations or loadings. Significant anthropogenic loadings of Se occurred in only two lakes and of Cr in only one lake. Crocket and Kabir (1981) found elevated Se and As concentrations together with enrichment of sediments by Cu and Ni. They attributed this to the occurrence of Se and As with Ni–Cu sulphide ores. In this study, Se and As concentrations did not correlate with Cu and Ni concentrations. However, their lakes were much closer to Sudbury smelters. In this study, higher surface concentrations of Cr, As, and Se usually were associated with higher background concentrations. Therefore, variations in these three trace elements likely reflected local geology with superimposed anthropogenic inputs at relatively low levels which were difficult to detect.

Relation of Anthropogenic Loadings to Precipitation Inputs

Anthropogenic loadings to sediments of undeveloped lakes are assumed to be largely attributable to atmospheric sources. Minor, local perturbations would not produce significant, sustained increases above background concentration in sediment cores. Some of the study lakes had cottages, specifically Manitou, Windfall, Ashby, Ardoch, and Antoine, and most lakes have some outboard motor activity. This potential for contamination by Pb from gasoline must have been minor in

TABLE 6. Comparison of trace element loadings in precipitation (P) with average anthropogenic (A) loadings which are present minus background loadings for all lakes. Standard errors of means are given in parentheses. Determination of trace element averages in precipitation is described in the text.

Trace element	Loading	Group			All groups
		NW	Central	SE	
Cu	A	−0.18	0.12	0.03	0.05
		(0.13)	(0.039)	(0.011)	(0.018)
	P	0.34	0.32	0.38	0.35
		(0.06)	(0.07)	(0.07)	(0.02)
Ni	A	−0.075	0.098	0.026	0.045
		(0.035)	(0.024)	(0.008)	(0.013)
	P	0.029	0.076	0.080	0.075
		(0.006)	(0.011)	(0.007)	(0.007)
Zn	A	0.07	0.67	0.77	0.57
		(0.037)	(0.208)	(0.221)	(0.131)
	P	0.47	0.87	0.86	0.91
		(0.083)	(0.102)	(0.063)	(0.054)
Pb	A	0.21	0.61	0.83	0.60
		(0.103)	(0.163)	(0.267)	(0.134)
	P	0.27	0.58	0.80	0.66
		(0.052)	(0.013)	(0.112)	(0.050)
Cd	A	0.006	0.013	0.014	0.012
		(0.0017)	(0.0037)	(0.0049)	(0.0025)
	P	0.007	0.019	0.016	0.018
		(0.0014)	(0.0014)	(0.0020)	(0.0012)
Cr	A	0.073	0.057	0.030	0.045
		(0.045)	(0.026)	(0.014)	(0.0153)
	P	—	—	—	0.030
Hg	A	0.0009	0.0009	0.0010	0.0010
		(0.00017)	(0.00025)	(0.00026)	(0.00013)
	P	—	—	—	0.003−0.006
As	A	0.012	0.034	0.033	0.027
		(0.0012)	(0.0140)	(0.0080)	(0.0055)
	P	—	—	—	0.063
Se	A	0.010	0.0	0.033	0.016
		(0.0088)		(0.019)	(0.0090)
	P	—	—	—	0.019

this set of lakes because there was no pattern of higher Pb in sediments with greater human activity.

The probability of detecting anthropogenic loadings of these trace elements is related to the magnitude of precipitation loadings (Table 6) relative to background loadings to sediments (Table 3). Precipitation loading of Pb was one order of magnitude greater than background loading to sediments which explains why anthropogenic loadings of Pb were readily detected in all of the cores examined. Precipitation loadings of Zn, Cd, Hg, and As (Group 2) were 1−3 times background loadings to sediments and similar increases in present loadings were observed. Therefore, statistically significant anthropogenic loadings were observed in the majority of cores. Although precipitation and anthropogenic loadings of Cr were similar, the increase relative to background loading was so low (about 14%) that detection of anthropogenic loadings of Cr would be difficult. These were detected in only two lakes in this study. Similarly there was little evidence for widespread or substantial anthropogenic input of Ni and Se because precipitation loadings were relatively low compared with background loadings.

Anthropogenic loadings of Cu, Ni, Zn, Pb, and Cd to sediments and precipitation loadings were compared in the three regions. Adequate precipitation data were not available for an interregional comparison of precipitation loadings of Cr, Hg, As, and Se, but loadings generally applicable to Ontario were calculated (Table 6). Loadings in precipitation of Ni, Zn, Pb, and Cd were significantly lower ($P < 0.05$) in the northwestern region, approximately 40−50% of central and southeastern region loadings. Anthropogenic loadings of Zn, Pb, and Cd to sediments were lower and not detected in the case of Ni in the northwestern group. Ni loading to sediments was highest in the central group of lakes. In contrast, Cu loading in precipitation was relatively uniform among regions. Anthropogenic loadings of Cu to sediments were relatively low or undetected in all regions.

The similarity of precipitation and anthropogenic sedimentary loadings was greatest for Pb and Se, still rather close for Ni, Zn, Cd, Cr, and As, and low for Hg and Cu. Regional differences in Pb and Cd loadings in precipitation were most

closely reflected in anthropogenic loadings. Precipitation and anthropogenic loadings of Ni were in closest agreement in the central group where loadings were highest. Zn loadings were closest in the central and southeastern groups. Anthropogenic loadings of Hg were much lower than precipitation loadings, probably due to scarcity of data on precipitation loadings of Hg. However, Cu in precipitation, which was measured adequately, is not reflected by increased loadings to sediments.

The potential for enrichment of surface sediments by Cu in precipitation was not realized in the majority of sediment cores, an observation made earlier in the Turkey Lakes system near Sault Ste. Marie, Ont. (Johnson et al. 1986). Possibly less Cu is removed to sediments or it is not held as effectively as other trace elements. The magnitude of this potential increase, which was at least as great as background concentrations (Table 6), and good precision in analysis of sediment Cu should have allowed detection of precipitation inputs if retention by sediments had occurred. The ratio of emissions from human and natural sources give Cu a high potential for enrichment (Galloway et al. 1982). Therefore, the question arises why anthropogenic loadings of Cu in sediments are not larger and observed more frequently. Johnson et al. (1986) suggested that Cu may be so mobile that enrichment of lake sediment profiles has been detected near mining activities only. Jeffries and Snyder (1981) found higher Cu concentrations close to Sudbury smelters, and the reflection of these in sediment cores from nearby lakes was observed by Crocket and Kabir (1981).

The foregoing analysis supports the hypothesis that precipitation loadings account for the observed anthropogenic loadings of Zn, Pb, Cd, Hg, and As in sediments of these Ontario lakes. Sediment core data also support the hypothesis that substantial proportions of Zn, Pb, Cd, Hg, and As in precipitation are anthropogenic and not part of the natural, background flux of trace elements to the lakes. While anthropogenic loadings to sediments of Ni, Cr, and Se were detected in one half or fewer lakes at low levels relative to background loadings, the average loadings above background were similar to precipitation loadings.

Relation of Trace Element Loadings to Concentrations in Fish

Positive relationships between loadings of contaminants such as Pb, Cd, and Hg and their residues in fish were expected. In contrast, essential trace nutrients in ample supply such as Cu and Zn might be expected to occur at relatively constant levels in fish independent of ambient loadings. The results provided some support for this hypothesis. Concentrations of Cu and Zn were approximately constant in spite of variation in levels of exposure, although there was variability among fish species. In contrast, Hg and Cd in fish were correlated with loadings to sediments for pooled species and some species alone. Intercepts were not significantly different from zero and increasing loadings of Hg and Cd contributed to increasing concentrations in fish. Cr and Se concentrations in fish were related to loading rates with significantly positive slopes but their regressions had relatively large intercepts, an intermediate response between those expected for trace nutrients and contaminants. The data suggested that Cr and Se were micronutrients in restricted supply. Pb in fish was not correlated with loadings. There was sufficient variability in Pb loadings among lakes, with the third highest coefficient of variation in nine trace elements, to provide an adequate test. However, the biomagnification potential of Pb was the lowest of the nine trace elements examined, suggesting relatively short residence time in water or low uptake and/or high elimination rates in fish. Pb may have a much shorter half-life in fish than Hg and Cd but published results are highly variable (Niimi 1983).

Most trace elements had the same rank in concentration in fish as rank in loadings to sediments. Only Hg and Se were more abundant in fish, relative to other trace elements, than their rank in loadings. The concentration of Pb in fish was lower than expected on the basis of rank of Pb in trace element loadings. If trace element loadings to sediments are indicative of exposure, then bioaccumulation of these nine trace elements decreased in the order Hg $\gg$ Zn and Se $>$ Cu $>$ Ni Cd and As $>$ Cr and Pb. Because of apparently high bioaccumulation of Hg and Se, more information is needed on their loadings from the atmosphere to lakes than is being collected in contemporary research programs.

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Recent Growth, Biochemical Composition, and Prey Field of Larval Haddock (*Melanogrammus aeglefinus*) and Atlantic Cod (*Gadus morhua*) on Georges Bank[1]

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A transect across southern Georges Bank in May 1983 showed higher levels of available prey for haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) larvae at two stratified sites than at a well-mixed site. At the stratified sites, prey biomass was high (30–300 μg dry wt·L^{-1}) near the surface above the thermocline; values were lower and more uniform with depth (10–30 μg dry wt·L^{-1}) at the well-mixed site. Larval population centers generally coincided with prey biomass vertically. Recent growth in dry weight of haddock larvae as estimated by RNA–DNA ratio analysis was higher at the stratified sites (8–13%·d^{-1}) than at the well-mixed site (7%·d^{-1}). Larvae appeared to be in excellent condition at the stratified sites, but up to 50% of haddock larvae from the well-mixed site had RNA–DNA ratios in the range observed for starved larvae in the laboratory. Cod collected at the same site were in better condition and growing faster than haddock. The data support the hypotheses that (1) stratified conditions in the spring favor good growth and survival of haddock larvae and (2) cod larvae are better adapted to grow and survive in well-mixed waters at lower levels of available food than haddock larvae.

Un transect traversant le sud du banc Georges en mai 1983 a montré une plus grande disponibilité des proies pour les larves d'aiglefin (*Melanogrammus aeglefinus*) et de morue (*Gadus morhua*) dans deux sites stratifiés que dans un site bien brassé. Dans les sites stratifiés, la biomasse des proies était élevée (30–300 μg·L^{-1} en poids sec) près de la surface, au-dessus de la thermocline; les valeurs étaient plus faibles et plus uniformes à mesure que la profondeur augmentait (10–30 μg·L^{-1} en poids sec) au site bien brassé. En général, les noyaux des populations de larves coïncident verticalement avec ceux de la biomasse des proies. Le taux de croissance en poids sec des larves d'aiglefin, tel qu'estimé par l'analyse du ratio ARN–ADN était plus élevé aux sites stratifiés (8–13 %·j^{-1}) qu'au site bien brassé (7 %·j^{-1}). Les larves des sites stratifiés semblaient être en excellent état, contrairement à celles du site bien brassé où jusqu'à 50 % des larves d'aiglefin recueillies avaient un ratio ARN–ADN se situant dans la plage des valeurs observées chez les larves affamées en laboratoire. Les larves de morue prélevées au même endroit étaient en meilleur état que celles de l'aiglefin et avaient également un taux de croissance plus rapide. Les données corroborent les hypothèses selon lesquelles 1) la stratification printanière favorise un bon taux de croissance et de survie des larves d'aiglefin et 2) les larves de morue sont mieux adaptées que celles de l'aiglefin pour croître et survivre dans des eaux bien brassées où les proies sont nombreuses.

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Understanding and predicting the variability in recruitment are among the major problems of fisheries science and fisheries management. While it is thought that variability in the physical environment is a major cause of recruitment variability, this linkage is largely based on empirical observations of correlations between an environmental variable such as temperature or wind stress and recruitment or a proxy such as catch (Sissenwine 1984). Little is known of the possible mechanisms involved. Progress has been limited by the complexity of the physical and biological systems and the limited resources and analytical tools available to the investigator. Here, we look at the relations among the physical environment, prey availability, and growth of haddock and cod larvae on Georges Bank, factors that may ultimately affect recruitment of these species.

Haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) are closely related species in the family Gadidae, exploited by sport and commercial fisheries on both sides of the Atlantic. Reductions in Georges Bank haddock spawning stock since the late 1960's have been associated with low recruitment and increased variability in year-class size (Clark et al. 1982). Cod, also heavily exploited during the same period, have been more successful in maintaining their numbers

[1]MARMAP Contribution No. MED/NEFC 84-36.

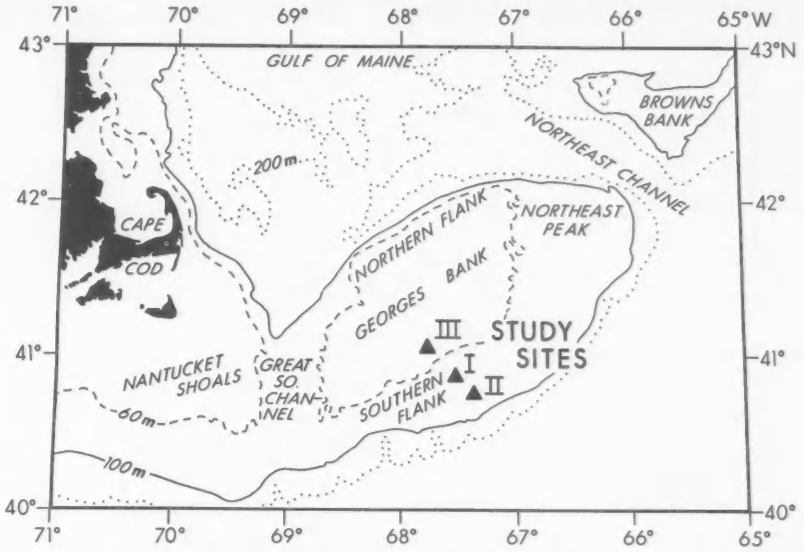

FIG. 1. Location of the three study sites along the southern flank of Georges Bank occupied during the R/V *Albatross IV* Cruise 83-03, 9–20 May 1983.

(Hennemuth et al. 1980). Both species have been categorized as shelf–shoal gyre, spring spawners (Sherman et al. 1984).

The principle spawning area of haddock and cod on Georges Bank is the northeast part. Peak spawning time of both species is in the spring, with cod preceding haddock by about 1 mo, although there is considerable overlap in their spawning seasons. The pelagic eggs and larvae are transported to the southwest in the clockwise mean flow around the bank. Concentrations of larvae of both species are found in April and May along the southern flank of Georges Bank between the 60- and 100-m isobaths (Smith et al. 1979; Lough 1984). The demersal 0-group fish are most abundant 6–8 mo later on the northern and eastern sections of Georges Bank (Grosslein and Hennemuth 1973; Overholtz 1985). In the spring a seasonal thermocline develops in the deeper water surrounding Georges Bank, while the shallower central portion, less than 60 m deep, tends to remain well mixed. Lough (1984) hypothesized that spring thermal stratification serves to concentrate prey organisms and larvae in the region of the thermocline. In May 1981, higher than average prey densities (factor of 5–10) were observed at thermocline depth (10–20 m) where fish larvae were also concentrated (Lough 1984). Both larvae and their prey were dispersed through the water column by a storm, reaggregating at and above the thermocline after 1–2 wk. The geography and hydrography of Georges Bank appear to provide several distinct environments for development of cod and haddock, and a mechanism to transport and retain the planktonic early life stages on the Bank.

Our objective was to compare and contrast three study sites occupied in May 1983 on the southern flank of Georges Bank from the well-mixed waters on the crest of the bank to the shelf–slope front near the 100-m isobath in order to elucidate the relation among the physical environment, larval prey field, and recent growth (<1 wk) of larval haddock and cod as determined by RNA–DNA ratio analysis. This effort was part of a larger study conducted on the R/V *Albatross IV* Cruise 83-03 to determine the fine-scale vertical and horizontal distribution of cod and haddock larvae and their prey organisms and to investigate the physical and biological processes responsible for their distributions.

Materials and Methods

Field Sampling

Larval fish and zooplankton prey organisms were collected on the R/V *Albatross IV* Cruise 83-03, 9–20 May 1983. Overall sampling strategy and the full array of sampling gear and instrumentation used have been described by Lough (1984). After locating a high concentration of larvae along the southern flank of Georges Bank, three time-series stations (Fig. 1) of 1–2 d each were made on a transect across the middle of the concentration: Site I — 40°54.3′N, 67°31.8′W, bottom depth 66–79 m (13–14 May 1983); Site II — 40°46.8′N, 67°23.7′W, bottom depth 88–93 m (15–16 May 1983); Site III — 41°41.7′N, 67°46.2′W, bottom depth 34–54 m (16–17 May 1983).

A vertical profile of fish larvae and zooplankton was made at each site every 6 h using an electronically controlled multiple opening/closing net and environmental sampling system (MOCNESS, Wiebe et al. 1976) equipped with different-sized underwater nets. The 1-m MOCNESS used to sample fish larvae has an effective mouth opening of 1 m^2 when towed at near 2 knots (3.7 $km \cdot h^{-1}$) and nine nets of 333-μm mesh which open and close sequentially on command. This net system was employed to sample discrete depths at 10-m intervals to within 5 m of the bottom, each net filtering ~250 m^3 of water integrating the 10-m stratum. Temperature data are obtained simultaneously with other sampling parameters. A more limited collection of water bottle samples was obtained for salinity depth profiles. Cod and haddock larvae to be used for biochemical analysis only were obtained from the last net which integrated the entire water column. Other sampling objectives and limited

TABLE 1. Summary information of the 1- and 0.25-m MOCNESS tows made at the three study sites on Georges Bank, May 1983. D = day; N = night.

Site	1-m MOCNESS (333-μm mesh)	Time of tow (E.D.T.)	Date	No. depth levels sampled	0.25-m MOCNESS (64-μm mesh)	Time of tow (E.D.T.)	Date	No. depth levels sampled
I	412 N	00:53–01:21	13	7	—	—	—	—
	414 D	11:39–12:14	13	7	—	—	—	—
	416 D	19:16–19:50	13	7	—	—	—	—
	417 N	22:10–22:43	13	7	—	—	—	—
	418 N	01:03–01:38	14	7	422 D	09:06–09:20	14	7
	421 D	06:32–07:08	14	7	424 D	14:47–14:58	14	6
	423 D	12:37–13:08	14	7	425 D	15:29–15:43	14	7
	426 D	18:39–19:12	14	7	427 N	21:00–21:12	14	7
II	428 N	01:01–01:42	15	8	429 N	04:02–04:19	15	8
	430 D	06:42–07:19	15	8	431 D	09:47–10:01	15	8
	432 D	12:44–13:23	15	8	433 D	15:34–15:55	15	8
	434 D	18:46–19:25	15	8	435 N	21:42–21:56	15	8
	436 N	00:53–01:37	16	8	437 N	03:00–03:18	16	8
III	438 D	07:55–08:21	16	5	439 D	10:42–10:49	16	5
	440 D	12:52–13:18	16	5	441 D	15:52–15:59	16	4
	442 D	18:29–19:05	16	5	443 N	21:30–21:36	16	4
	444 N	01:14–01:39	17	5	445 N	03:47–03:54	17	4

manpower precluded saving additional larvae from the vertical samplers for RNA–DNA ratio analysis. To obtain a wider size range, larger postlarval cod were sampled for biochemical analysis from one double oblique 10-m MOCNESS tow (3-mm mesh) at Site III. A sufficient number of postlarval haddock were not collected for analysis. The larvae collected for biochemical analyses were all removed individually from the sample prior to routine preservation, placed in petri dishes, frozen, and analyzed ashore. The prey field of the larval fish was sampled with the 0.25-m MOCNESS, which had nine nets of 64-μm mesh. The same sampling profile was used for the 0.25-m MOCNESS as for the 1-m MOCNESS, except that in this case each net filtered ~25 m^3 of water within a 10-m stratum. All net plankton samples were preserved in 4% formaldehyde–seawater solution after a subsample was taken for silhouette zooplankton analysis. A summary of the MOCNESS tow information at each of the three sites is provided in Table 1.

Laboratory Processing and Analysis

Since the fish used for biochemical analysis came from integrated water-column tows and the RNA–DNA ratio reflects growth conditions over the last several days, data on depth distribution of larvae and their prey were treated to provide composite or integrated measures of abundance. In the laboratory all fish larvae were removed from the preserved MOCNESS samples, identified, and their standard length measured to the nearest 0.1 mm for a complete length–frequency sample. The preserved length of cod and haddock were corrected for shrinkage to live length by the method described in Bolz and Lough (1983) and the data grouped into 1-mm size classes and standardized to number per 100 m^3. The mean number of individuals per species was calculated for each depth by summing across the same depth level for all tows made at that site. Average integrated water column abundance (number per 10 m^2) also was calculated for each study site. A composite length–frequency distribution was made for each species at each site by summing all the tow data. Differences among the length–frequencies on each site were tested for similarity of curves by the Kolmogorov–Smirnov (KS) test and for differences among population mean length by the Students' *t*-test (Sokal and Rohlf 1969). Temperature and salinity data also were averaged across all tow depth levels at a site and the 95% confidence limits estimated. Density (sigma-*t*) values were calculated where sufficient temperature and salinity data permitted.

Biochemical analysis of larvae was completed in the laboratory within 2 mo after the cruise. Fish were freeze-dried and weighed to the nearest microgram. Larvae smaller than 0.8 mg dry wt from a single tow were pooled by size to give a minimum dry weight of 0.8 mg which is the lower limit of sensitivity for the analytical methods used. A 0.8-mg cod or haddock larvae would be about 4 wk old. Pooled samples consisted of two or three fish of similar size. All fish greater than 0.8 mg dry wt were analyzed individually. Samples were homogenized in ice-cold water to give a final tissue concentration of about 0.75 $mg \cdot mL^{-1}$. Nucleic acids were extracted and partially purified from 1.4 mL of homogenate using a modification of the Schmidt–Thannhauser method (Munro and Fleck 1966; Buckley 1979). RNA and DNA were determined from the absorbancy at 260 nm of the appropriate hydrolysates. Protein was determined on duplicate 0.05- or 0.10-mL samples of homogenate using a modification of the Lowry method (Hartree 1972).

Whereas haddock and cod larger than 3 mg dry wt (about 12 mm standard length) were sampled for biochemical analysis at Site III, certain of the data analyses as indicated in the text were limited to fish less than 3 mg dry wt. This was done to maintain a more uniform size range between sites and to minimize any possible effects of size on estimated growth rate. Extensive laboratory studies have shown no effect of size on the relationship between RNA–DNA ratio, temperature, and growth rate of prerecruits up to 3 mg dry wt (Buckley 1982, 1984; Buckley et al. 1984). Few data are available on fish greater than 3 mg dry weight reared under controlled conditions.

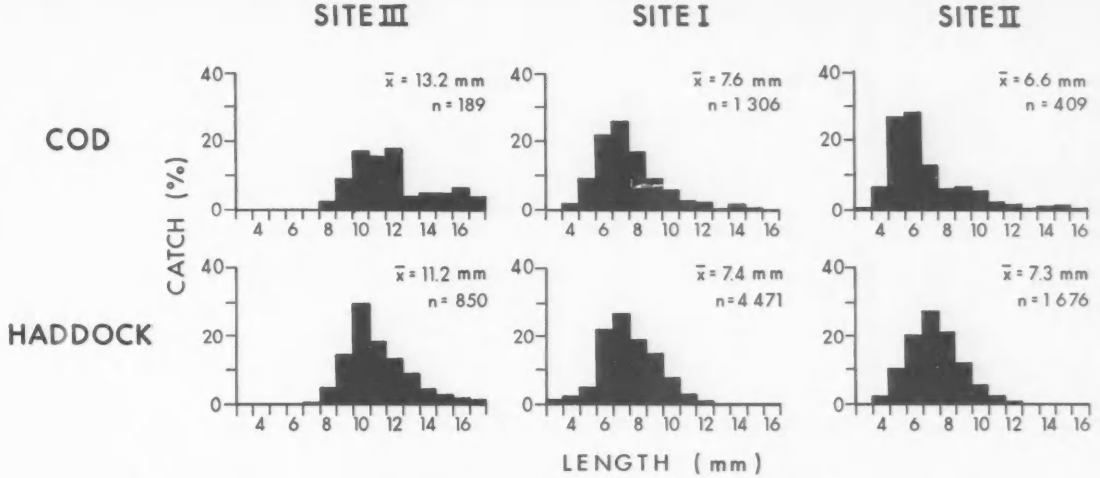

FIG. 2. Composite length–frequency distributions of cod and haddock larvae collected at the three sites.

Prey Biomass Index

Silhouette photography (Lough and Potter 1983) was used at sea for the rapid identification and enumeration of zooplankton samples from the 0.25-m MOCNESS. Although the technique has some limitations in the level of identification, results are obtained with minimal effort and are available for real time use. An index of the availability of prey items to larval fish at each of the three study sites was derived from the estimated abundances of copepod nauplii and the copepodite and adult stages of *Pseudocalanus* sp., *Centropages typicus*, and *Calanus finmarchicus*. This is consistent with the results of Kane (1984), who reported that the bulk of the diet of larval cod and haddock on Georges Bank consisted of these copepod life stages. In this study, copepod eggs were not treated as separate food items because *Pseudocalanus* sp. eggs, which accounted for the majority of eggs found in the samples, are coincidentally ingested with the females, who retain their eggs until hatched (Corkett and McLaren 1978). The relative contribution of copepod eggs to the prey biomass is small, accounting for <14% of the total gut biomass (wet weight) (Kane 1984).

The prey biomass index, expressed as micrograms dry weight per litre, was made by summing the biomass estimates of individual prey life stages for each sample (net) to produce a vertical profile for a MOCNESS tow. This was done by multiplying the standardized number per litre of a life stage times its biomass (micrograms wet weight) estimated from a width–weight equation developed by Pearre (1980). Prey widths were taken from literature values reported by Davis (1984) and Cohen and Doyle (1984), or if not available, measurements were made directly from the samples. The wet weight was multiplied by 0.277 to convert to dry weight (Pearre 1980). Prey biomass estimates were summed across the same depth level for all tows made at the same site and a mean density and coefficient of variation (CV) calculated.

Copepod nauplii could not be adequately identified to stage or species from the silhouette photographs. All nauplii were assigned an average biomass of 0.75 μg dry wt, equivalent to *Pseudocalanus* sp. fourth stage nauplii (0.11-mm width). Other potential sources of error in our estimates of prey biomass are extrusion of the smallest copepod nauplii through the 64-μm mesh and consumption and digestion of zooplankters not included in the index, such as lamellibranch larvae. Both of these sources of error should be small, since lamellibranch larvae, the most abundant noncopepod taxa of a suitable size, are found intact throughout the larval gut and are presumed to provide relatively little nutritional value and copepod nauplii <70 μm were virtually absent in the larval gadid diet (Kane 1984; Tilseth and Ellertsen 1984).

Results

Distribution and Abundance of Larvae and Prey

The highest abundance of larvae in May 1983 was located on the central, southern part of the flank (Site I), consistent with the southwest drift of eggs and larvae from the spawning ground on northeastern Georges Bank. Haddock larvae were more abundant than cod on all sites. The composite length–frequency distributions of cod and haddock at the three sites are shown in Fig. 2. Both cod and haddock had a modal length of 7 mm at Sites I and II on the flank, but at Site III on the shoals, the size of cod increased to 13.2 mm and haddock to 11.2 mm. There was no significant difference ($P < 0.05$) between the length–frequency distributions of cod and haddock at Sites I and II using the KS test. However, using the *t*-test there was a significant difference ($P < 0.05$) between the mean size of cod at Sites I and II (7.6 versus 6.6 mm). The length–frequency distributions between Sites I and III and between II and III, flank versus shoal sites, were found by both statistics to be highly significantly different ($P < 0.01$) for both cod and haddock. That is, on average, larger and presumably older larvae were found on shoal Site III.

The water column temperature and salinity at Site III were uniformly 8.9°C and 33.06 psu, indicative of the well-mixed waters atop Georges Bank (Fig. 3). At Sites I and II, along the deeper, southern flank, stratification of the surface water was apparent, primarily due to the increasing seasonal insolation. Surface temperature was near 9°C and decreased to 6°C below 30 m depth. A shallow thermocline was observed with the greatest variability in temperature observations at 20–30 m. Salinity was slightly less in the surface 30 m but only varied in

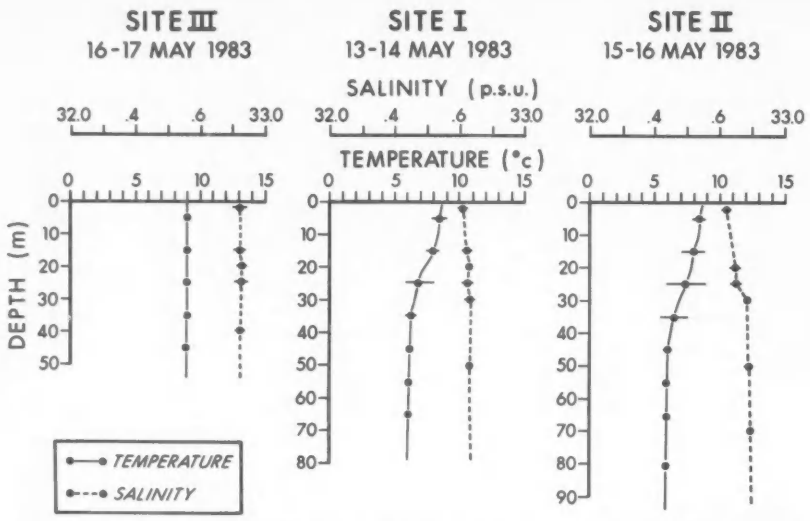

FIG. 3. Mean temperature and salinity profiles at the three study sites on Georges Bank. The horizontal bar at each depth level represents the 95% confidence limits.

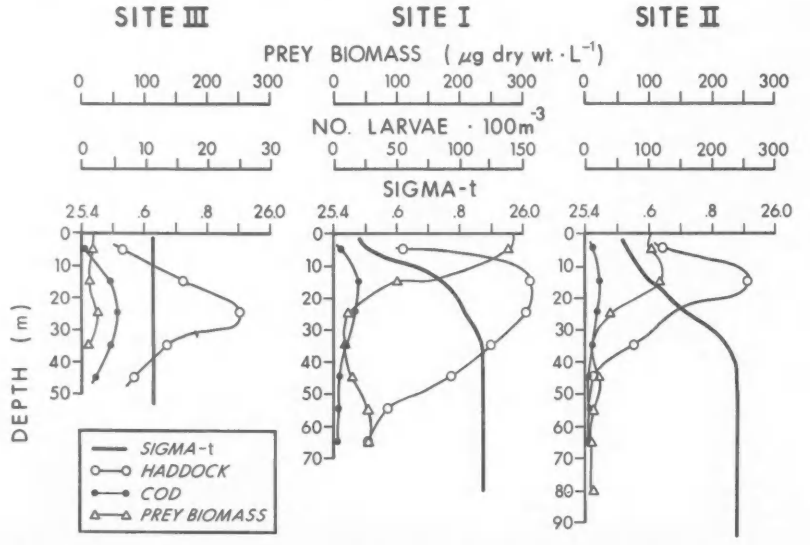

FIG. 4. Sigma-*t* profile and vertical distribution of haddock and cod larvae and their prey at the three study sites on Geoges Bank. Note difference in scale for density of larvae for Sites I, II, and III.

the range of 32.8−33.0 psu. Sigma-*t* values plotted in Fig. 4 correspond with the temperature profiles and illustrate the well-mixed Site III (sigma-$t \simeq 25.63$) versus the stratified Sites I and II. On Site I and II, less dense water (sigma-$t \cong 25.5$) at the surface increased in density (sigma-$t > 25.9$) to about 30 m depth and thereafter was essentially constant to bottom. Since temperature accounted for the greatest part of the density change with depth at Sites I and II, we refer to this water column structure as a thermocline.

Both cod and haddock larvae had similar vertical distribution patterns on a site, but the peaks were more pronounced for haddock than cod (Fig. 4). At Site III, in the well-mixed water, the relatively low numbers of larvae were found throughout the water column from near bottom to surface; however, most of the larval population resided between 10 and 40 m depth. The highest mean density of cod and haddock occurred at 20−30 m. On both Sites I and II, in stratified water, more than 90% of the larval population resided in the upper 50 m of the water column. At Site I, larvae were broadly distributed through the water column with the highest mean densities located between 10 and 40 m depth. At Site II, near the shelf/slope-water front, the highest mean density of larvae was located at 10−20 m, in the central region of the thermocline.

The dominant copepod species enumerated from the Georges

TABLE 2. Prey biomass indices and larval cod and haddock abundance in the upper 50 m of the water column at three study sites on Georges Bank, May 1983.

Site	No. depth levels (0–50 m)	Mean prey biomass (μg dry wt$\cdot L^{-1}$)	Range	ISD	CV (%)	Integrated prey biomass (μg dry wt$\cdot m^{-2}$)
			Zooplankton			
I	5	90	18–278	110	121	4.52
II	5	59	12–117	49	83	2.96
III	4	17	10–25	7	40	0.66
		Mean larval density (no.$\cdot 100\ m^{-3}$)				Integrated abundance (no.$\cdot 10\ m^{-2}$)
			Cod			
I	5	11.8	5.8–20.1	6.5	55	59.0
II	5	6.3	0.3–11.6	4.1	65	31.6
III	4	3.5	0.3–5.7	2.2	63	17.3
			Haddock			
I	5	115.6	58.9–160.2	43.2	37	577.9
II	5	60.7	4.2–127.4	45.6	75	303.3
III	4	13.8	6.4–24.9	7.3	53	69.2

TABLE 3. RNA concentration and RNA–DNA ratio of haddock larvae <3 mg dry wt caught with the 1-m MOCNESS. Values in the same column without a letter in common are significantly different (Duncan's new multiple-range test; $P \leq 0.05$).

MOCNESS	Site	*N*	RNA ($\mu g \cdot mg^{-1}$)	RNA–DNA ratio
412	I	33	41.9±4.5 a	4.44±0.57 bc
414	I	30	40.3±3.7 a	5.05±0.69 a
430	II	9	41.7±2.5 a	4.67±0.29 ab
432	II	9	43.5±3.1 a	4.24±0.31 c
434	II	29	42.7±4.7 a	4.37±0.51 bc
438	III	15	35.3±4.7 b	3.76±0.37 d
440	III	20	34.2±3.1 b	3.36±0.37 e
442	III	11	36.2±3.0 b	3.63±0.43 de

Bank study sites were *Calanus finmarchicus*, *Pseudocalanus* sp., *Centropages typicus*, and *Oithona similis*. The copepodite and adult stages of *Oithona* are believed to successfully avoid capture and do not comprise a large part of the larval diet (Kane 1984; Cohen and Lough 1983). The depth profiles of the prey biomass index shown in Fig. 4 are the mean of four hauls at Sites I and III and five hauls at Site II. At Sites I and II the prey biomass index was high (30–300 μg dry wt$\cdot L^{-1}$) near the surface above the thermocline and particularly so at Site I where *Calanus* had the highest abundance. The prey biomass index at Sites I and II declined with depth to a low below the thermocline, but showed a slight increase around 50 and 60 m depth. On Site III the prey biomass index was generally lower and more uniform with depth (10–25 μg dry wt$\cdot L^{-1}$) and appeared to have a maximum at middepth (20–30 m).

The peak densities of larvae generally coincided with the highest prey biomass indices vertically on all three sites, although at Site I the larvae were broadly distributed in the water column below the peak prey biomass located in the surface 20 m. Since greater than 90% of the larval population is located above 50 m depth on Georges Bank, a comparison of the prey biomass available to the larvae was made for the upper 50 m of the water column at the three sites (Table 2). More than 80% of the prey biomass resides in the surface 50 m. Site I on the midflank of Georges had the highest index of mean prey biomass (90 μg dry wt$\cdot L^{-1}$), which was comparable with the somewhat lower mean biomass index (59 μg dry wt$\cdot L^{-1}$) at the deeper Site II. The shoal Site III had the lowest mean biomass index (17 μg dry wt$\cdot L^{-1}$), about one fourth the available prey biomass found at the deeper sites. Integrated prey abundance indices (micrograms dry weight per square metre) showed the same relative difference in prey biomass among the three sites. Variability in the prey biomass with depth was greatest at Site I (CV = 121%) and Site II (CV = 83%) in stratified waters, but considerably less at Site III (CV = 40%) as expected for well-mixed waters. Variability in the larval mean density was similar (CV = 37–75%) for all three sites.

Biochemical Composition of Larvae

Significant differences in dry weight and biochemical composition of haddock collected by the 1-m MOCNESS were observed between net hauls and between sites (Tables 3 and 4). RNA concentrations (micrograms per milligram dry weight) were lower and DNA concentrations similar or higher in haddock taken at Site III than at either Site I or II (ANOVA, $P < 0.05$). Protein concentrations of haddock were similar at all three sites. The RNA–DNA ratios of haddock at Sites I and II were significantly higher than those of haddock at Site III. Analysis of data from the individual MOCNESS hauls indicated that haddock from the three oblique tows made at Site III had uniformly lower RNA concentrations and RNA–DNA ratios than samples from Sites I and II. At Site I, the only site where a night tow was used for biochemical analysis, no significant difference was observed in RNA concentration of larvae taken in day and night tows, while the RNA–DNA ratio was significantly higher (ANOVA, $P < 0.05$) in day-caught

TABLE 4. Dry weight and macromolecular composition of haddock collected at the three study sites with the 1-m MOCNESS. Data on dry weight are mean and standard deviation of individual larvae. Data on chemical composition are mean and standard deviation of individual samples. *F* ratio is the treatment mean square divided by the error mean square from analysis of variance. Each site was considered a treatment. $*P \leq 0.05$.

	All larvae				Larvae < 3 mg				Larvae 1–3 mg			
	I	II	III	*F*	I	II	III	*F*	I	II	III	*F*
Number of larvae	89	75	65		89	72	46		32	16	46	
Number of samples[a]	63	47	65		63	47	46		32	16	46	
Dry weight (mg)	0.86±0.58	0.92±0.90	2.65±1.27	85.79*	0.86±0.58	0.79±0.62	2.01±0.49	76.64*	1.49±0.44	1.73±0.65	2.02±0.49	10.41*
RNA concentration (μg RNA·mg dry wt^{-1})	41.1±4.2	42.7±4.1	35.1±3.5	61.57*	41.1±4.2	42.7±4.1	35.0±3.6	48.02*	41.2±4.5	42.7±2.80	35.0±3.6	36.07*
DNA concentration (μg DNA·mg dry wt^{-1})	8.86±1.67	9.76±1.19	9.77±1.40	7.81*	8.84±1.71	9.76±1.19	10.24±2.55	7.93*	8.93±1.56	9.63±1.12	9.97±1.37	5.17*
Protein concentration (μg protein·mg dry wt^{-1})	439±60	413±58	426±48	3.21	438±63	413±58	432±91	1.75	451±50	434±35	422±49	3.55
RNA–DNA ratio	4.73±0.69	4.40±0.46	3.64±0.46	64.70*	4.73±0.69	4.40±0.46	3.55±0.42	61.17*	4.68±0.69	4.46±0.37	3.55±0.42	49.22*

[a]Larvae less than 0.8 mg dry wt were pooled to give this minimum sample size.

than in night-caught larvae (Table 3). At Site III, the only site where sufficient numbers of both cod and haddock were sampled for biochemical analysis, the mean RNA–DNA ratio of cod (4.56) sampled by the 1-m MOCNESS (Table 5) was greater than the mean RNA–DNA ratio of haddock (3.64) (ANOVA, $P < 0.05$). When the data were split by size, no significant difference was observed in the RNA–DNA ratios of cod and haddock greater than 3 mg dry wt, while a significant difference was observed for larvae less than 3 mg dry weight (ANOVA, $P < 0.05$). The distributions of RNA–DNA ratios of haddock collected at Site III (Fig. 5) indicate that about 50% were in poor condition (RNA–DNA ratio < 3.5) and 5% were in extremely poor condition (RNA–DNA ratio < 3.0), indicative of starvation. Laboratory studies have shown 3.2–3.5 to be the minimum RNA–DNA ratio of fed cod and winter flounder reared at 7–8°C (Buckley 1979, 1980, 1981). Larvae starved in the laboratory 2 d or longer had values less than 3.5. In contrast with Site III, no haddock collected at Site II had a RNA–DNA ratio less than 3.5 and only 5% of individuals collected at Site I had values less than 3.5 (Fig. 5). The low RNA–DNA ratios observed at Site III represent one of the relatively few reports of finding starved larvae at sea (O'Connelll 1980; Buckley 1984; Theilacker 1986).

Highly significant correlations were observed between dry weight and RNA, DNA, and protein content (micrograms per larva) of cod and haddock at all three sites (Table 6). This is expected, since the protein, RNA, and DNA content of fish increase in proportion to the dry weight during the larval period (Buckley 1981). No significant correlation between dry weight and RNA–DNA ratio of haddock was observed at any of the three sites as reported by Lough (1984). This lack of a correlation between dry weight and RNA–DNA ratio precluded the use of analysis of covariance, with dry weight as the independent variable or covariate, to evaluate differences in RNA–DNA ratio between sites. It further indicated that differences in larval size between sites had no effect on larval RNA–DNA ratios. A correlation between dry weight and DNA concentration (micrograms per milligram dry weight) was observed for haddock at Site III. Dry weight was correlated with both nucleic acid and protein concentration of cod collected at Site III.

To further ensure that the differences in biochemical composition observed between haddock larvae sampled at Sites I, II, and III reflected differences in the condition of fish between sites rather than differences in size, the data were analyzed for selected size ranges. Altogether, three sets of analyses were done: (1) all haddock sampled from the 1-m MOCNESS, (2) haddock less than 3 mg dry wt, and (3) haddock between 1 and 3 mg dry wt (Table 4). In all cases the results were similar; the RNA–DNA ratio of haddock was significantly lower (ANOVA, $P < 0.05$) at Site III than at either Site I or II. The third analysis (haddock between 1 and 3 mg dry wt) gave a similar size range of individuals at all three sites.

Growth of Larvae at the Three Study Sites

The recent growth rates of larvae can be estimated from the RNA–DNA ratio and water temperature using an equation derived from data on eight species of temperate marine fish including both haddock and cod reared in the laboratory (Buckley 1984). Unlike most other techniques available for estimation of larval growth rates, RNA–DNA ratio analysis strongly reflects growth conditions over the last 2–4 d prior to

TABLE 5. Dry weight and macromolecular composition of cod collected at Site III with the 1- and 10-m MOCNESS. Data are the mean ± 1 SD. All samples consisted of an individual fish.

	Captured with 1-m MOCNESS	Captured with 10-m MOCNESS	All cod
Number	19	35	54
Dry weight (mg)	2.95±1.61	38.2±17.8	25.8±22.2
RNA concentration (μg RNA·mg dry wt^{-1})	38.96±3.88	36.74±4.38	37.52±4.31
DNA concentration (μg RNA·mg dry wt^{-1})	8.69±1.30	5.51±0.93	6.63±1.86
Protein concentration (μg protein·mg dry wt^{-1})	485±41	603±98	561±100
RNA–DNA ratio	4.56±0.69	6.74±0.63	5.97±1.23

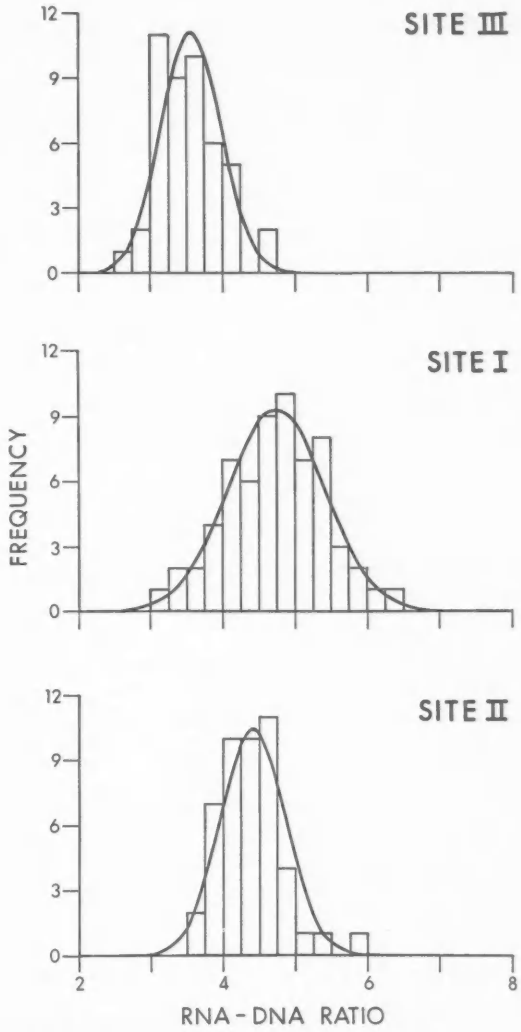

FIG. 5. Distributions of RNA–DNA ratios of haddock observed at three sites on the southern flank of Georges Bank. The curves describe normal distributions.

sampling. Since estimates of recent growth based on RNA–DNA ratio analysis are temperature dependent and since the exact location of haddock collected at Sites I and II relative to the thermocline is unknown, growth rates were estimated for larvae above and below the thermocline (Table 7). At Site II, however, 90% of the larvae appeared to reside above the thermocline and would be growing at the faster rate. It is uncertain whether young haddock could use the thermocline to some metabolic advantage, as suggested for some species of zooplankton which reside in deeper colder waters after feeding in warmer surface waters (McLaren 1963). Recent growth of haddock less than 3 mg dry wt was faster at the stratified Sites I and II than at the well-mixed Site III. At Site III where both cod and haddock were sampled, growth of larvae less than 3 mg dry wt was considerably higher for cod (10.6%·d^{-1}) than for haddock (7.1%·d^{-1}).

Discussion

The mean size and range of larval haddock and cod sampled with 1-m MOCNESS were different at the three sites. Haddock collected at Sites I and II were on average smaller than those collected at Site III (Table 4; Fig. 2). Haddock and cod taken at Site III were older than those collected at Sites I and II based on otolith ageing (G. Bolz, NMFS, Woods Hole, MA, unpubl. data). These observations are consistent with the known spawning area of haddock and cod on the northeast part of Georges Bank and the residual drift on the bank which is described as a semienclosed clockwise gyre (Lough 1984).

It is well established that size or stage of development can affect the biochemical composition of fish (Love 1970). Previous studies have shown that RNA–DNA ratios increase with increasing larval age or size (Buckley 1982; Buckley et al. 1984). No similar correlation was observed for haddock sampled at any of the three sites with the 1-m MOCNESS, probably because of the limited size range of individuals collected. The more rapid removal by predation of the weaker, slower growing fish at sea (Wyatt 1972; Jones 1973; Hunter 1976; Frank and Legget 1982; Fortier and Leggett 1984) and differences in prey availability to different size larvae at sea and in the laboratory could also help explain these observations. The correlations observed between size and biochemical composition (RNA, DNA, and protein concentration and RNA–DNA ratio) of cod larvae and pelagic juveniles sampled with the 1- and 10-m MOCNESS probably resulted from the larger size range of individuals sampled (0.7–73.1 mg).

TABLE 6. Regression parameters and relations between dry weight and chemical composition of cod and haddock collected at the three study sites. All data are for linear regressions. The slope (m) and the elevation (b) are given only for significant correlations ($P \leq 0.05$).

Dependent variable	Site I haddock ($N = 63$)			Site II haddock ($N = 47$)			Site III haddock (<3 mg dry wt, $N = 46$)			Site III haddock (all dry weights, $N = 65$)			Site III cod (all dry weights, $N = 54$)		
	r	m	b	r	m	b	r	m	b	r	m	b	r	m	b
[RNA] ($\mu g \cdot mg\ dry\ wt^{-1}$)	−0.02	—	—	−0.06	—	—	−0.13	—	—	−0.09	—	—	−0.47	−0.10	39.89
[DNA] ($\mu g \cdot mg\ dry\ wt^{-1}$)	−0.04	—	—	−0.16	—	—	−0.40	−1.11	12.21	−0.28	−0.31	10.60	−0.77	−0.06	8.30
[Protein] ($\mu g \cdot mg\ dry\ wt^{-1}$)	0.22	—	—	0.21	—	—	0.21	—	—	0.16	—	—	0.51	2.30	502.02
RNA ($\mu g \cdot fish^{-1}$)	0.98	40.64	0.30	0.99	41.67	0.82	0.92	33.82	2.19	0.98	33.86	2.83	0.98	33.40	61.76
DNA ($\mu g \cdot fish^{-1}$)	0.94	8.60	0.22	0.98	9.02	0.61	0.84	7.97	3.77	0.96	8.92	1.77	0.97	4.66	19.48
Protein ($\mu g \cdot fish^{-1}$)	0.98	462.06	−17.92	0.98	462.06	−17.92	0.93	473.11	−98.73	0.97	483.18	−21.17	0.98	610.06	−141.26
RNA · DNA^{-1}	0.00	—	—	0.13	—	—	0.34	0.29	2.96	0.11	—	—	0.72	0.04	4.93
Protein · DNA^{-1}	0.24	—	—	0.35	3.25	39.37	0.49	6.95	28.96	0.34	1.97	39.13	0.89	1.20	60.81
RNA · $protein^{-1}$	−0.30	0.0071	0.1022	0.34	−0.0061	0.1107	−0.35	−0.0067	0.0971	−0.27	−0.0018	0.0875	−0.76	−0.0004	0.0802

The consistently lower RNA−DNA ratios of all size groups of haddock larvae at Site III indicate that haddock larvae were in poor condition at Site III compared with either Site I or II. Further, this difference in condition appears to be independent of differences in age or size of larvae at the three sites, since no correlation was observed between size and RNA−DNA ratio of haddock. Any attempt to adjust the observed RNA−DNA ratios for differences in larval size between sites would magnify the differences observed between the shoal Site III and the deeper Sites I and II, since when observed, the relation between size, or age, and RNA−DNA ratio is positive during the larval stage.

Differences in the larval prey field between sites are the most likely explanation for the differences in condition and growth of haddock larvae between sites. The relative difference in the prey biomass index among the three study sites corresponds to the same trend in larval RNA−DNA ratios or growth rates, their values being similarly high at Sites I and II but significantly lower at Site III. One would expect Site I with the highest prey biomass index to offer the highest potential for larval growth which is borne out by the highest mean RNA−DNA ratio of 4.7 and a growth rate of $12.7\% \cdot d^{-1}$. One also would expect Site III to offer the lowest growth potential (RNA−DNA = 3.55, growth rate = $7.1\% \cdot d^{-1}$) since the *Calanus* nauplii and early copepodite stages are an order of magnitude lower in abundance here. Note that at Site I, where haddock larvae were widely distributed through the water column and where the preferred prey biomass index showed the greatest variability with depth (Fig. 4; Table 2), the RNA−DNA ratios of haddock showed the broadest distribution (range 3.15−6.26, CV = 14.6%). At Site II, where haddock appeared to be concentrated at the thermocline in an area of high prey availability, their RNA−DNA ratios were more uniform (range 3.59−5.98; CV = 10.5%). At Site III, where prey densities were uniformly low with depth, the RNA−DNA ratios of larval haddock (range 2.75−4.59; CV = 12.6%) were lower and more uniform than at Site I. The observations that the RNA concentrations were lower and the DNA concentrations similar, or higher, in haddock sampled at Site III than at either Site I or II support our observation that there was more food available to larval haddock at the stratified Sites I and II than at the shoaler, well-mixed Site III. These observations also support the hypothesis that the poor condition of haddock larvae was due to low food availability. Similar trends in nucleic acid concentration have been observed fo cod larvae reared at different levels in the laboratory (Buckley 1979).

The most obvious physical difference between sites was the presence of a thermocline between about 20 and 30 m at Sites I and II and the isothermal conditions existing at Site III. During late spring and summer, stratification occurs throughout the deeper waters of the continental shelf and is a factor in the maintenance of high levels of biological productivity in this region (O'Reilly and Busch 1984; Townsend et al. 1984). Chlorophyll concentrations and some herbivorous copepods, especially *Calanus finmarchicus*, are located near or above the seasonal pycnocline but considerable variability has been observed (Lough 1984; Sameoto 1984; Townsend et al. 1984).

Starvation and predation are considered the primary causes of mortality prior to recruitment (Hunter 1976). The role of food availability in larval population dynamics is most often considered in the context of starvation during the "critical period" which occurs at the time of yolk-sac exhaustion (for a review see May 1974). However, for certain species including

TABLE 7. RNA–DNA ratio and estimated growth (Gpi) of haddock and cod less than 3 mg dry wt at three sites on Georges Bank.

Site	N	RNA–DNA ratio	Gpi[a] (%·d^{-1})		Age at 3 mg dry wt (days post-hatch)	
			6°C	9°C	6°C	9°C
I: haddock	63	4.73	9.9	12.7	38	30
II: haddock	47	4.40	8.3	11.1	46	34
III: haddock	46	3.55	—	7.1	—	53
III: cod	12	4.29	—	10.6	—	36

[a]Gpi is the mean daily protein growth rate. During the larval stage when the protein concentration remains relatively constant, the rate of increase of protein is very close to that of dry weight. Growth at 6 and 9°C is for fish below and above the thermocline at Sites I and II.

cod and haddock, an equally critical role of food availability may be its effect on growth rate and the duration of the larval and juvenile stages which in turn affect total mortality during these early life history stages. Laboratory studies (Lasker et al. 1970; Laurence 1974, 1978; Houde 1978; Laurence et al. 1981; Solberg and Tilseth 1984) together with field (Bolz and Lough 1983; Buckley 1984) and enclosure studies (Ellertsen et al. 1981; Gamble and Houde 1984) have demonstrated that larval growth is highly variable and dependent on temperature and food availability. Since growth rate determines the duration of the early life history stages, total mortality is strongly dependent on larval growth rate and food availability. For example, the number of days required for haddock larvae to reach 3 mg dry wt can be estimated from the average dry weight of haddock at hatching (68.1 μg, Laurence 1974) and the growth rates of haddock at Sites I, II, and III (Table 7). At 9°C the length of time required to reach 3 mg dry wt was 30, 34, and 53 d, respectively, at the growth rates estimated for Sites I, II, and III. Assuming a constant mortality rate of 7%·d^{-1} (Lough 1984), survival to 3 mg dry wt would vary by a factor of 5 between sites. The effect of food availability on larval survival may be further compounded by size-specific mortality and increased predation on weaker, slower growing larvae (Rosenberg and Haugen 1982; Bailey and Yen 1983).

Histograms of RNA–DNA ratios of individuals collected at a site can provide some insight into the importance of predation and starvation mortality. If, for example, as observed for cod larvae in May 1981 (Buckley 1984), the distribution is truncated above a minimum RNA–DNA ratio for starved larvae, predation on slower-growing larvae is suggested. RNA–DNA ratios of haddock collected in May 1983 at Site I were broadly and normally distributed, showing no indication of truncation (Fig. 5). Values observed at Site III were narrowly distributed and somewhat truncated at the point where starvation mortality would occur, suggesting light predation pressure. Values observed at Site II were more narrowly distributed than at Site I, possibly due to a better match between vertical distribution of larvae and their prey at Site II. All values were above the level where starvation mortality is indicated. Confirmation of these predictions of predation pressure would require actual estimates of predator abundance which were not obtained on the cruise.

The higher RNA–DNA ratios and estimated growth rate of cod larvae compared with haddock larvae at Site III are consistent with previous laboratory, field, and modelling studies. Experiments of direct competition between larval cod and haddock reared together at different plankton densities have shown that daily specific growth rates were 2–2.5 times greater for cod than haddock (Laurence et al. 1981). When cod and haddock larvae were reared separately, growth rates of the two species were similar (Laurence 1978). Results from stochastic models of food-limited growth indicate that haddock are considerably more food limited than cod during the larval stage (Laurence 1985). The faster growth of cod at Site III probably resulted from their ability to utilize a greater size spectrum of the zooplankton prey assemblage than haddock (Kane 1984). The similar RNA–DNA ratios and estimated growth rates of cod and haddock >3 mg dry wt suggest that any competitive advantage of cod may be limited to younger, smaller stages. These data are consistent with the hypothesis that cod larvae are better adapted for growth and survival in well-mixed water at low levels of available food than haddock larvae.

In our evaluation of larval prey field we have categorized potential food items as either available or unavailable based primarily on their size and presence or absence in larval stomach contents. Zooplankton that remained intact throughout the larval gut were also considered unavailable. Further work is needed on the digestibility and nutritional value of various larval prey items such as lamellibranch larvae and phytoplankton which are commonly found in the larval gut. The behavioral and physical mechanisms of both predator and prey which result in either selection or rejection of particular prey items also deserve further study.

RNA–DNA ratio analysis provides an index of condition and recent growth rate of fish. It gives the field ecologist a valuable tool to evaluate the response of an organism to its environment and should facilitate establishing the linkage between environmental conditions and recruitment variability. In this study, good agreement was observed between the relative availability of prey at the study sites and the RNA–DNA ratio or estimated growth rate of larval haddock. Our results provide support for the hypotheses that (1) stratified conditions in the spring favor good growth and survival of haddock larvae and (2) cod larvae are better adapated to grow and survive in well-mixed waters at lower levels of available food than haddock larvae.

The recruitment pattern of the Georges Bank gadid stocks may reflect the adaptation of the larvae to their environment (Lough 1984, p. 427–428). Cod larvae, more adapted to winter conditions when prey densities are lower, have a relatively low but stable recruitment pattern (Hennemuth et al. 1980). Haddock larvae, in contrast, are more adapted to spring condi-

tions and may require the higher prey densities following stratification of the water column. The extremely variable nature of haddock recruitment, only 3 or 4 good years out of 20, may ultimately be related to the dynamics and variability of the spring stratification process on the flank of Georges Bank.

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Recruitment to Northwest Atlantic Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) Stocks: Influence of Stock Size and Climate

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Koslow, J. A., K. R. Thompson, and W. Silvert. 1987. Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate. Can. J. Fish. Aquat. Sci. 44: 26–39.

Year-class success of both Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks in the northwest Atlantic exhibits large-scale coherence and low-frequency variability with apparent periodicities of 10–20 yr. Several physical and biological variables in the region exhibit similar large-scale coherence and apparent periodicity. Multiple regression analysis indicates that year-class success in northwest Atlantic cod stocks tends to be associated with large-scale meteorological patterns and offshore winds. Recruitment to most haddock stocks from the Scotian Shelf to Georges Bank is negatively associated with abundance of 0-group mackerel, which may be due to predation over winter and/or to a combination of environmental features including sea-surface temperature, large-scale atmospheric pressure systems, and freshwater outflows. Statistical analyses often did not define a unique set of variables that best predicted fishery recruitment due to widespread intercorrelations among environmental processes and the likelihood that not all relevant processes entered directly into the analyses. There is little evidence that stock reproductive output during the study period was significantly related to year-class success.

Le taux de réussite des classes d'âge des populations de morue (*Gadus morhua*) et d'aiglefin (*Melanogrammus aeglefinus*) du nord-ouest de l'Atlantique montre une cohérence à grande échelle et une variabilité de faible fréquence pour des périodicités apparentes de 10 à 20 ans. Plusieurs variables physiques et biologiques de la région montrent une cohérence à grande échelle et une périodicité apparente similaires. L'analyse de la régression multiple révèle que le taux de réussite d'une classe d'âge chez les populations de morue de nord-ouest de l'Atlantique a tendance à être associé à des configurations météorologiques à grande échelle et à des vents de terre. Le recrutement de la plupart des populations d'aiglefin du plateau continental scotian jusqu'au banc Georges est négativement associé avec l'abondance du maquereau du groupe 0, ce qui peut être causé par la prédation pendant l'hiver et/ou par une combinaison de caractéristiques environnementales, notamment la température de la mer en surface, les systèmes de pression atmosphérique à grande échelle et les débits sortants d'eau douce. Les analyses statistiques n'ont généralement pas défini de série unique de variables qui prédisaient le mieux le recrutement d'une population en raison d'intercorrélations répandues entre les phénomènes environnementaux et du fait que tous les phénomènes pertinents n'entraient vraisemblablement pas directement dans les analyses. Peu de faits prouvent que le rendement reproducteur d'une population pendant la période d'étude était relié de façon significative au taux de réussite d'une classe d'âge.

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In recent decades, recruitment has been highly correlated among Atlantic cod (*Gadus morhua*) stocks spawning on the offshore banks from west Greenland to the Scotian Shelf and among haddock (*Melanogrammus aeglefinus*) stocks spawning from the Scotian Shelf to Georges Bank (Fig. 1) (Koslow 1984). Significant correlations are also found between recruitment to cod and haddock stocks in the region. These patterns suggest that gadoid recruitment in the northwest Atlantic is influenced by large-scale biological and perhaps climatic features.

Although the marine climate of the northwest Atlantic is far from being well understood, analyses to date indicate that several major physical variables vary on scales similar to the gadoid recruitment patterns. Loucks et al. (1986) performed principal component analysis (PCA) on monthly mean sea-surface temperature (SST) data from southern Greenland to the mid-Atlantic Bight (1948–80). Half the variance in the data set was explained by the first two components, which had length scales of 1500 and 500 km, respectively, and were centered in the region from the Grand Banks to the Gulf of Maine (Fig. 2). Koslow et al. (1986) performed a PCA on monthly air pressure data from 1950 to 1980 over the North Atlantic and eastern

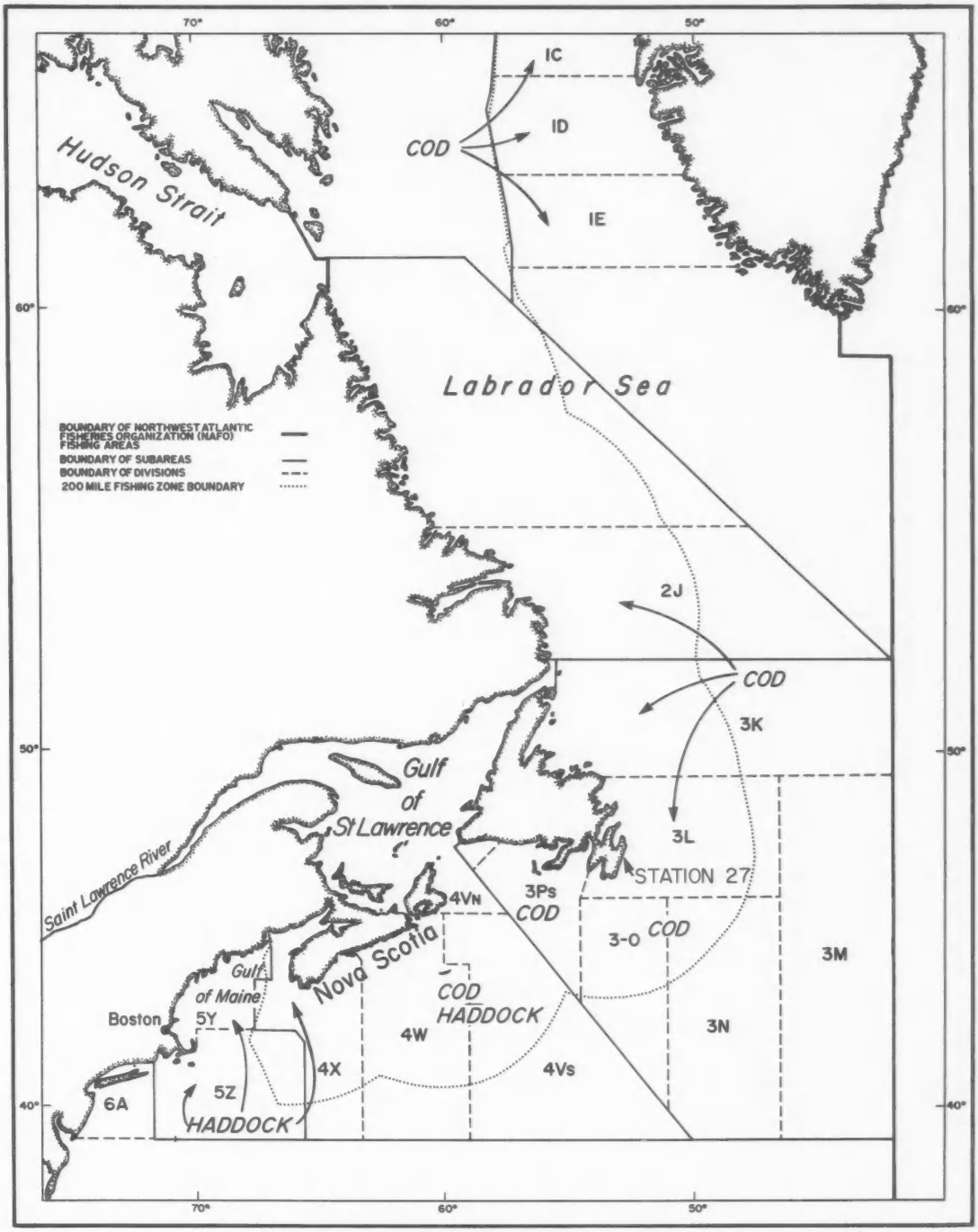

FIG. 1. Northwest Atlantic showing the location of fish stocks, sampling sites, and features discussed in the paper.

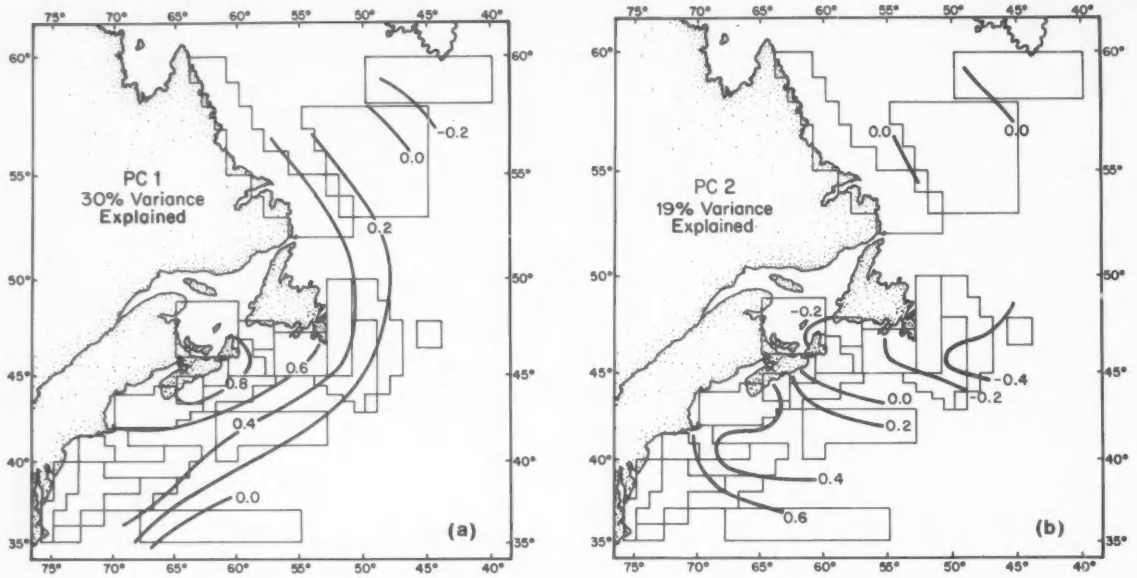

FIG. 2. Northwest Atlantic (from Loucks et al. 1986) showing the 24 subareas into which sea surface temperature (SST) observations were seasonally averaged. The contours of correlations of the winter SST time series with winter principal components (PC) (a) 1 and (b) 2 are shown and explain 30 and 19% of the variance in winter SST. The contours are qualitatively similar for other seasons.

North America (Thompson and Hazen 1983) and found 66% of the variance explained by the first four components, each of which represents a regional pattern of atmospheric variability (Fig. 3). Precipitation over eastern North America, which is related to regional wind and atmospheric pressure as well as to regional runoff patterns (Koslow et al. 1986), is coherent from the St. Lawrence drainage basin to New England (Vines 1984). Thus, an overview of factors affecting northwest Atlantic climate indicates that the spatial scale of their variability is on the order of that found for gadoid recruitment.

However, the history of correlation analyses between fisheries and physical variables in the northwest Atlantic is more noted for its failures than its successes. Chase (1955) proposed that recruitment to Georges Bank haddock was regulated by transport of the larvae offshore, as indicated by a correlation with an atmospheric pressure index representing cross-shelf winds, but the relationship subsequently broke down (Grosslein and Hennemuth 1973). Wright et al. (1986) subsequently showed that alongshore winds more effectively regulate drift in the region. Sutcliffe et al. (1977) noted that landings for a number of species in the Gulf of Maine were correlated with both SST and St. Lawrence River outflow. The river discharge was considered a primary driver of the system, as indicated by lagged correlations with SST from the Gulf of St. Lawrence to the Gulf of Maine, which they hypothesized were based upon the freshwater influence on the stability and summer warming of the upper mixed layer (Sutcliffe et al. 1976). However, more recent analyses indicate that St. Lawrence discharge and SST downstream are both primarily regulated by large-scale atmospheric processes; the river/SST correlation may be largely spurious (Koslow et al. 1986). Sutcliffe et al. (1983) proposed that the basis for a negative correlation between St. Lawrence discharge and Labrador Shelf cod recruitment was the inhibitory influence of freshwater-induced stratification on nutrient mixing into the upper mixed layer. Precipitation over the adjacent St. Lawrence and Hudson Bay watersheds was assumed to be correlated, but this is contradicted by analysis of regional rainfall patterns (Fortin et al. 1983). Using partial correlation analysis, Koslow (1984) showed that in any case, Labrador cod recruitment was more closely related to salinity variations in the central Labrador Sea, which Lazier (1980) had suggested were caused by large-scale changes in North Atlantic atmospheric pressure patterns and exchange between Arctic and Atlantic waters. Exchange between relatively low-salinity, low-nutrient shelf water and high-salinity, high-nutrient slope water has been implicated in regulating the productivity of the Gulf of Maine and Scotian Shelf (Fournier et al. 1977; Houghton et al. 1978).

Predation by mackerel (*Scomber scombrus*) has been proposed as a mechanism underlying recruitment of cod and herring in the Gulf of St. Lawrence (Lett and Kohler 1976; Winters 1976; Lett 1978; Steele and Henderson 1984; but see Doubleday and Beacham 1982). Juvenile mackerel, which overwinter from the outer Scotian Shelf to south of Georges Bank, may also influence gadoid recruitment in these areas (Kulka and Stobo 1981). Young fish form a significant proportion of their diet at this time: 7–17% of their gut contents, by weight, with gadoids being identified as a major component (Kulka and Stobo 1981). Although other fish species, such as silver hake (*Merluccius bilinearis*) (Waldron 1983), as well as invertebrates, also prey on young gadoids, only juvenile mackerel are both sufficiently widely distributed and represented by adequate time series so their influence on recruitment can be tested.

In this paper, we explore the most likely causes of large-scale coherence in recruitment reported for gadoid stocks in the northwest Atlantic (Koslow 1984). Four distinct mechanisms

TABLE 1. Sources for data on year-class success (R) and stock reproductive output, which is calculated from data on numbers- (N) and weight-at-age (W), maturity ogives (M), and a fecundity–weight relationship. Fecundity (F) for cod stocks was $F = (0.48W - 0.12) \times 10^6$, where W is weight (kg) (May 1967). For haddock stocks, fecundity was taken as the mean of two relationships: (1) $F = 752\,000\,W^{1.647}$, where W is in kg (Hodder 1963), and (2) $F = 237W^{1.13}$, where W is in gm (Hislop and Shanks 1981). Stock designations refer to North Atlantic Fishery Organization (NAFO) areas (Fig. 1).

Stock	Data	Source
Cod 1	R,N,W,M	Sv. Aa. Horsted, NAFO SCR Doc. 82/VI/50
Cod 2J3KL	R,N,W	C. A. Bishop and S. Gavaris, NAFO SCR Doc. 82/IX/111
	M	Fleming 1960
Cod 3NO	R,N,W	C. A. Bishop and S. Gavaris, NAFO Res. Doc. 82/VI/57
	M	Fleming 1960
Cod 3Ps	R,N,W	C. A. Bishop and S. Gavaris, CAFSAC Res. Doc. 33/32
	M	Fleming 1960
Cod 4VsW	R,N,W	J. A. Gagne, A. F. Sinclair, and C. Dale, CAFSAC Res. Doc. 84/78
	M	Beacham 1983a
Haddock 4VW	R,N,W	D. E. Waldron, CAFSAC Res. Doc. 80/61; R. Mahon, P. Simpson, and D. E. Waldron CAFSAC Res. Doc. 83/47
	M	Beacham 1983b
Haddock 4X	R,N,W	R. O'Boyle, MFD, BIO, pers. comm.
	M	Beacham 1983b
Haddock 5Y	R, spawning stock	W. J. Overholtz, S. H. Clark, and D. Y. White, Woods Hole Laboratory Ref. Doc. 83-23.
Haddock 5Z	R,N,W,M	W. J. Overholtz, S. H. Clark, and D. Y. White, Woods Hole Laboratory Ref. Doc. 82-83; Clark et al. 1982
Mackerel	R	E. D. Anderson, Woods Hole Laboratory Ref. Doc. 83-40

will be examined — wind-driven advective losses, runoff-regulated nutrient dynamics, slope water inputs of nutrients, and predation — each with its own predictor: local wind, runoff, salinity, and juvenile mackerel. We consider temperature as well because of both its direct physiological influence and its association with a range of ecological processes. We also examine the relationship to fishery recruitment of large-scale, but less well-understood variables represented by principal components of SST over the northwest Atlantic (Fig. 2) and basin-wide atmospheric pressure patterns (Fig. 3). Although local conditions presumably directly regulate fishery ecology, the local variables at our disposal may not adequately represent the influence of large-scale climatic conditions on local marine environments. The relative influence of stock reproductive output on year-class success will also be examined.

Materials and Methods

All available time series for cod and haddock stocks in the northwest Atlantic were used (Table 1) that were of reasonable length for statistical purposes (approximately 15 yr) with the exception of two time series that appeared unrelated to others from the region: cod in the Gulf of St. Lawrence (4TVn), which has many features of a distinct inland sea, and in the Gulf of Maine (5Y), which is at the southern end of the cod's range (Fig. 1). Recruitment, the number of fish entering the fished populations, was obtained from cohort analyses and lagged back to the first year of life, when year-class strength is largely determined. The most recent years in the time series were truncated due to their unreliability in cohort analyses (Pope 1972). The recruitment time series were normalized using a $\log_{10}$ transformation.

The reproductive effort at age t (R_t) of each stock was calculated:

$$(1) \quad R_t = N_t W_t M_t F(W).$$

where N_t and W_t are the number and weight at age t in the stock, obtained from the cohort analysis, M_t is the proportion mature, and $F(W)$ is a weight-dependent fecundity function (see Table 1 for data sources). R_t was summed over all ages by year to produce time series of stock reproductive effort. The mean reproductive effort at age over the period was calculated to determine the age by which half of a population's reproductive effort was expended, which provides an estimate of the generation time of the stock. Although this method does not explic-

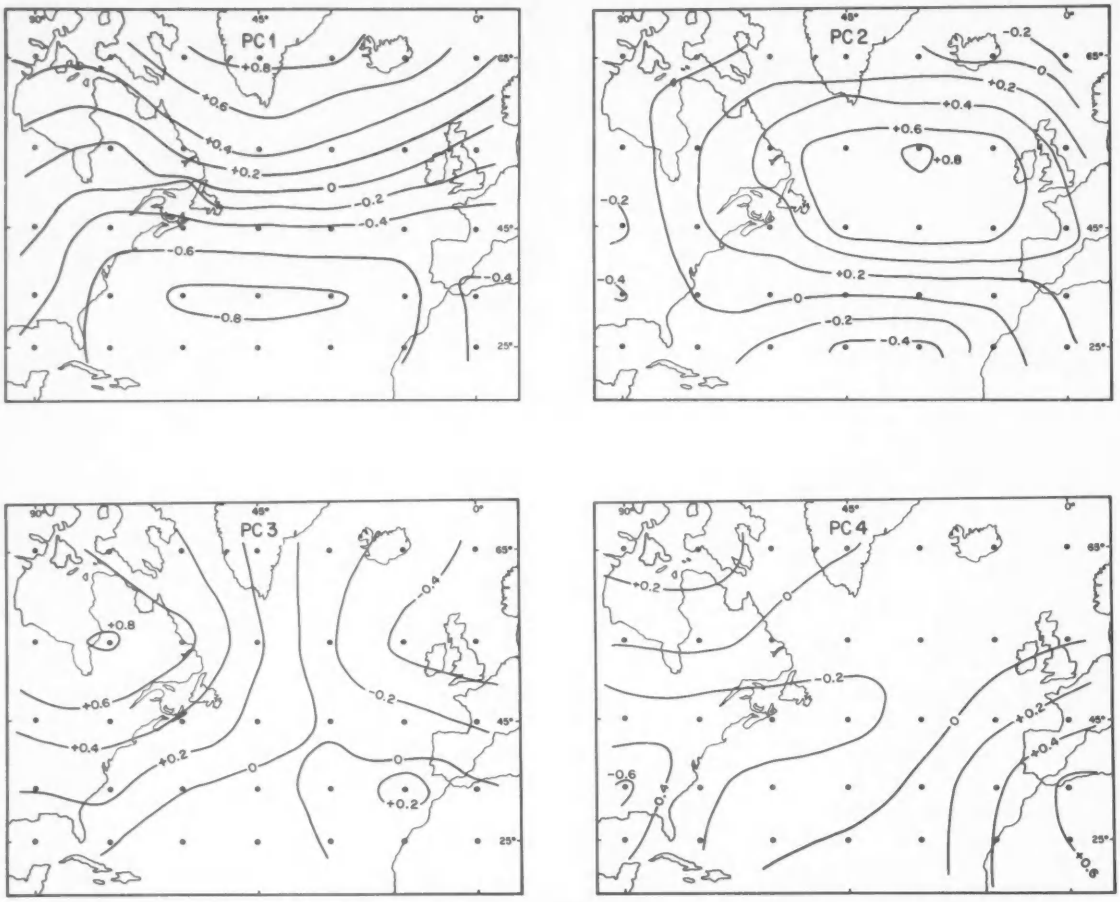

FIG. 3. Contours of correlations of principal components (PC) 1–4 of atmospheric pressure computed for a grid (shown) over the North Atlantic and eastern North America with atmospheric pressure at those grid sites. The PCs explained 33, 15, 13, and 8% of the variance in the data set, respectively, or 69% of the total variance.

itly take into account density-dependent changes in fecundity, data on changes in age-at-maturity and weight-at-age were used when such data were available.

Annual means were used in the analyses for all physical variables, since a critical period narrower than the first year of life cannot be defined for these stocks. Analyses were performed as well with seasonal means for these variables, but the results were not qualitatively different from those obtained with annual means, and the additional variables (each physical variable was now represented by four intercorrelated seasonal means) did not seem justified. However, the influence of higher-frequency events, such as storms, is not adequately represented by our method.

SST variation was examined from ship observations averaged over 24 areas in the northwest Atlantic corresponding to topographic and hydrographic features (Loucks et al. 1986) (Fig. 2). Local SST variables were based upon means over the area(s) where the fish stock is found during the first year of life. Large-scale processes related to SST variation were represented by the first two PCs for SST over the entire region (Loucks et al. 1986), which explained approximately 50% of the variance in the data set (Fig. 2). PC 1 corresponds to a coherent response in SST over virtually the entire continental shelf region. PC 2 provides an index of opposing SST conditions centered over the Grand Banks and Gulf of Maine/mid-Atlantic Bight regions (Fig. 2).

Geostrophic winds calculated by Thompson and Hazen (1983) for the Gulf of Maine, Scotian Shelf, Grand Banks, and Labrador Sea were used to examine the influence of local wind conditions upon year-class success of stocks within each of these regions. The most effective wind direction was determined from the coefficients from a multiple regression of the N–S and E–W components of local wind stress with recruitment to each stock. Results of these regressions (i.e. the most effective wind directions) were then used in subsequent analyses. The influence of large-scale atmospheric processes upon recruitment was examined using the first four PCs obtained from analysis of atmospheric pressure over a grid covering the entire North Atlantic and eastern North America (Fig. 3) (Koslow et al. 1986).

There are relatively few long-term time series of salinity in the region. Mean salinity over the upper 20 m at station 27 off

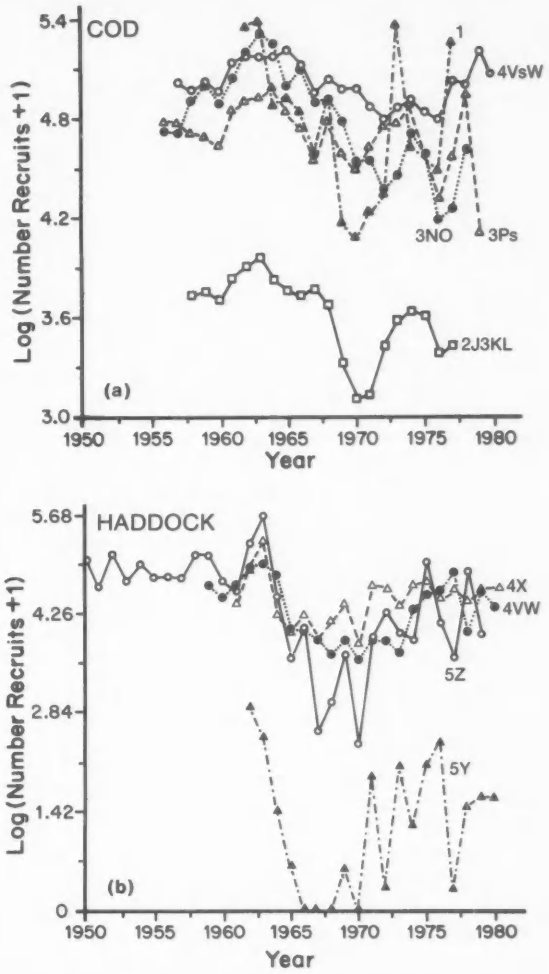

FIG. 4. Time series of recruitment lagged back to the year of spawning for (a) five cod and (b) four haddock stocks in the northwest Atlantic. For locations of stocks, see Fig. 1.

St. John's, Newfoundland, which is available for the entire period in question (Akenhead 1983), was used in relation to recruitment of stocks from the Labrador Shelf to the Scotian Shelf. A shorter time series for surface salinity at Boston (Sutcliffe et al. 1976) was used in relation to recruitment of stocks in the Gulf of Maine area. The influence of runoff was examined using the time series for St. Lawrence River outflow, as used by Sutcliffe et al. (1976, 1977) and updated courtesy of K. Drinkwater (Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S.). There are no adequate time series for runoff into the Labrador Sea, so analyses with Labrador Shelf and Grand Banks stocks were performed with data for the St. Lawrence River solely for purposes of comparison with Sutcliffe et al. (1983), since Fortin et al. (1983) indicated that precipitation over the St. Lawrence watershed, which is closely related to St. Lawrence outflow (Koslow et al. 1986), is not correlated with precipitation over the Hudson Bay watershed.

The primary methods of statistical analysis are autocorrelation analysis to examine time series for evidence of periodicity (Jenkins and Watts 1968), in which the significance of the autocorrelations at lag (L) is conservatively determined from the transformation $Z_L = r_L\,(n - L)^{1/2}$, where r is the autocorrelation and n is the number of data points in the time series (Davis 1973); correlation analysis, in which the degrees of freedom (and hence the significance) of the product-moment correlation coefficients (r) are routinely corrected for autocorrelation in the time series using the method of Garrett and Toulany (1981); and stepwise regression analysis, in which variables are entered or removed if significant at $p \leq 0.10$ or $p > 0.10$, respectively, to determine the significance of relationships between recruitment and various intercorrelated physical variables. Because the results of multiple regression analyses may be unstable when the "independent" variables are intercorrelated, the regressions were conducted stepwise both forward and backward. In those cases when the two methods produced different results, their explanatory powers were compared by determining the one for which

$$(2)\quad \frac{(1 - R^2)}{(n - k - 1)^2}$$

is a minimum, where R^2 is the variance explained, n is the number of data points, and k is the number of variables in the regression (Wonnacott and Wonnacott 1979). Since these methods reveal only linear relationships, scatterplots were examined for evidence of nonlinear relationships. Because the time series for 0-group mackerel and Boston salinity are shorter than for the other environmental variables, these variables were removed and the analyses rerun if they did not enter regressions significantly, in order not to lose degrees of freedom.

Results

Recruitment Time Series

The time series of year-class success (Fig. 4) were updated from those presented in Koslow (1984) and include data for an additional stock, Gulf of Maine (5Y) haddock. The series continue to exhibit coherence among stocks within each species as well as some degree of similarity between the two species, as previously reported. This can be seen as well from the consistently significant correlations of recruitment among stocks within each species and the several weak correlations observed for recruitment between cod and haddock stocks (Table 2). Interestingly, there is no tendency for stocks within particular geographic regions (e.g. the Labrador Sea, Grand Banks, Scotian Shelf, Gulf of Maine) to be more strongly correlated; rather, the relationships span their entire range from West Greenland to the Gulf of Maine.

The most striking feature of the recruitment time series, other than the degree of correlation among them, is the low-frequency quasi-periodicity that they apparently exhibit. The time series are too short to establish the nature of the periodicity, but autocorrelation analysis corroborates its presence, at least over the time period in question (Fig. 5). The apparent period length, which we define to be the lag between peaks in autocorrelation or twice the lag to the peak in negative autocorrelation, was generally between 10–20 yr for both the cod and haddock stocks, although it varied somewhat less among the haddock stocks (Table 3).

TABLE 2. Product-moment correlations of log-transformed year-class success among northwest Atlantic cod and haddock stocks. The time series are shown in Fig. 4. The degrees of freedom and significance levels are corrected for autocorrelation in the time series. ? = $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns = not significant.

	Cod					Haddock			
	1	2J3KL	3NO	3Ps	4VsW	4VW	4X	5Y	5Z
Cod									
1		0.73	0.40	0.56	0.54	0.52	0.38	0.46	0.35
2J3KL	*		0.78	0.66	0.63	0.53	0.24	0.53	0.23
3NO	ns	***		0.61	0.80	0.33	0.08	0.32	0.07
3Ps	*	**	**		0.24	0.07	0.17	0.38	0.08
4VsW	*	**	***	ns		0.41	0.02	0.15	−0.00
Haddock									
4VW	?	?	ns	ns	ns		0.62	0.68	0.58
4X	ns	ns	ns	ns	ns	**		0.80	0.70
5Y	?	*	ns	?	ns	**	***		0.76
5Z	ns	ns	ns	ns	ns	*	*	***	

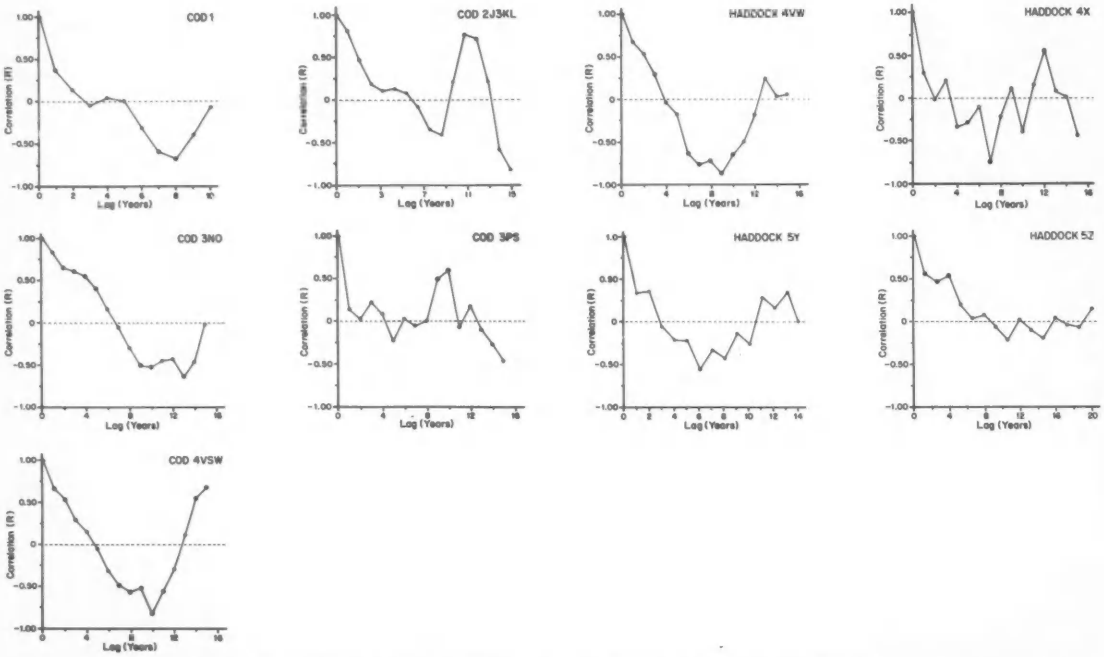

FIG. 5. Lagged autocorrelations for the cod and haddock recruitment time series (Fig. 4). The enlarged dots indicate autocorrelations significant at $p \leq 0.10$.

Stock and Recruitment

The appearance of apparent periodic fluctuations in all stocks examined further points to the importance of endogenous or exogenous processes in driving recruitment to northwest Atlantic cod and haddock stocks. We first examined the relationship of reproductive output in these stocks to recruitment because, as Ricker (1954) noted, a strongly domed stock–recruitment relationship can produce recruitment cycles with a period of twice the generation length. We examined this relationship both by plotting recruitment against the stock's expected reproductive output and by calculating the expected period length that would be produced by this mechanism (i.e. twice the time to maximum reproductive output, or effective generation time). This analysis also permits us to determine what relationship between stock reproductive output and recruitment should be taken into account when we next examine how environmental factors may affect recruitment.

Due to intensive fishing of these stocks and resulting reduction in their age structure, their effective generation length is

TABLE 3. Maximum significant autocorrelation, lag, and inferred period length for fishery recruitment, annual means of physical variables, and 2X the stock generation length. ? = $p \leq 0.10$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = not significant.

Variable	Autocorrelation (r)	Lag (yr)	Period (yr)	2X generation length (yr)
Recruitment				
Cod				
1	−0.68?	8	16	12
2J3KL	0.77*	11	11	11
3NO	−0.65*	13	26	9
3Ps	0.60*	10	10	13
4VsW	−0.81**	10	20	10
Haddock				
4VW	−0.86***	9	18	8
4X	−0.75**	7	14	10
5Y	−0.56*	6	12	—
5Z	ns	—	—	7
Physical				
SST				
PC 1	−0.75**	14	28	
PC 2	−0.56**	10	20	
NE Scotian Shelf	0.62**	9	9	
Air pressure				
PC 1	0.45*	11	11?	
PC 2	−0.51*	12	24	
PC 3	0.59*	18	18	
PC 4	0.40?	9	9	
Scotian Shelf				
N wind	−0.53*	10	20	
Station 27 salinity	ns	—	—	
St. Lawrence River	−0.79***	11	22	
Mackerel	−0.94**	10	20	

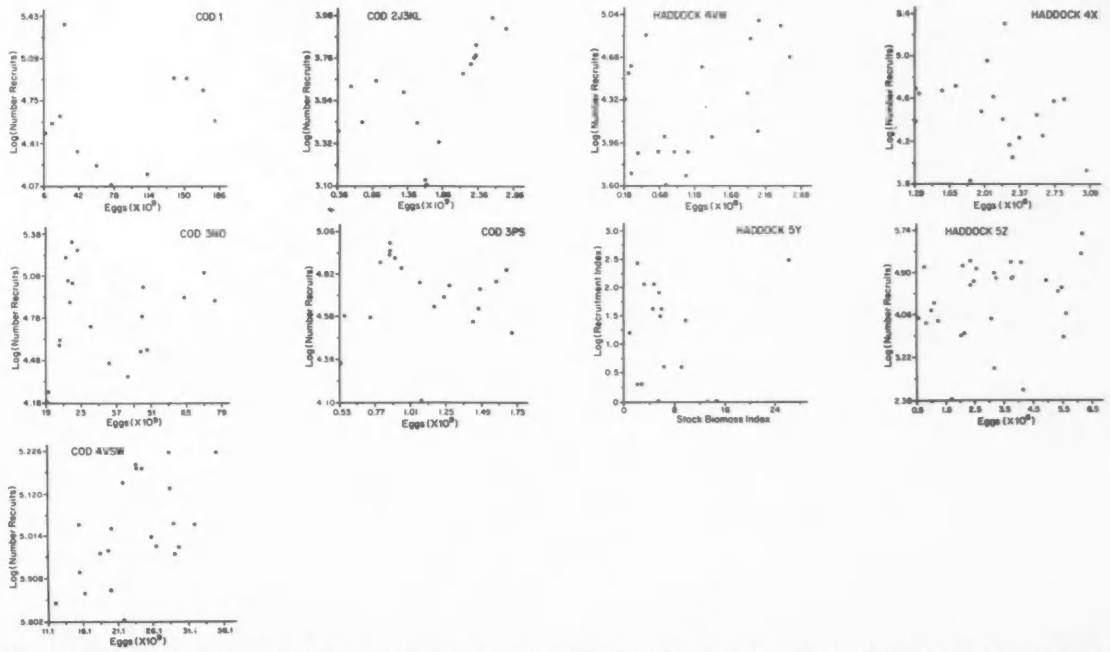

FIG. 6. Scatterplots of recruitment lagged back to the year of spawning with calculated reproductive output for that year (except for haddock 5Y, for which only an index of spawning biomass from research vessel surveys is available).

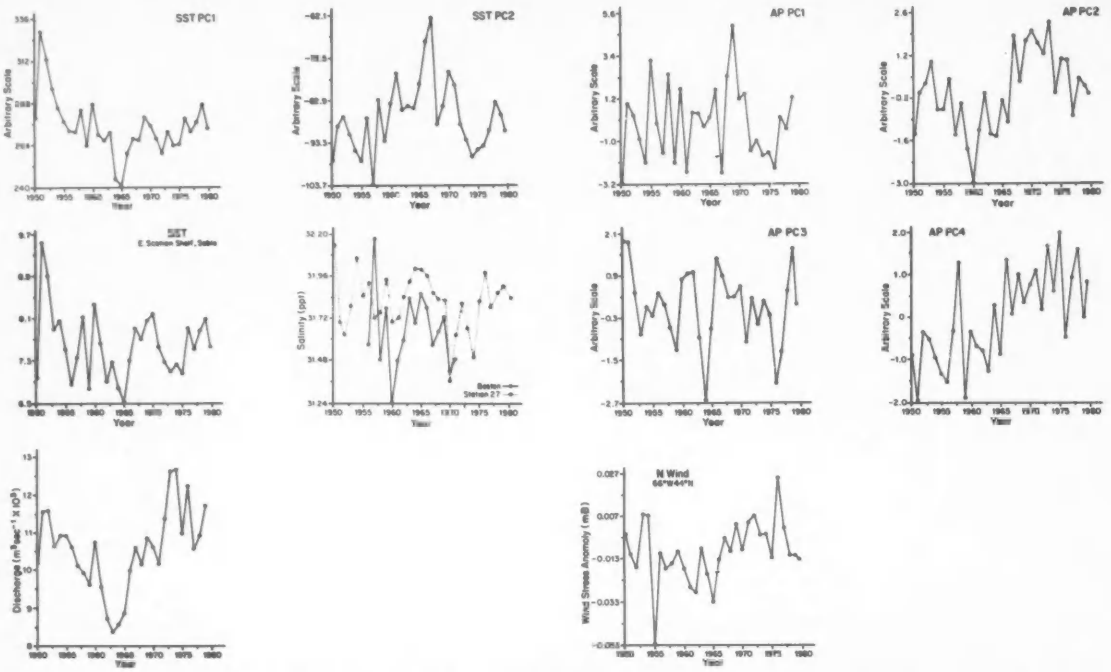

FIG. 7. Time series for environmental variables used in analyses with fishery year-class success: sea-surface temperature (SST) principal components (PC) 1 and 2 and on the eastern and central (i.e. Sable Is. Bank) Scotian Shelf; annual discharge from the St. Lawrence River; salinity at Boston and Station 27 off Newfoundland; air pressure (AP) PC 1–4; and the northerly (dominant) component of wind on the Scotian Shelf (66°W, 44°N). See text for further explanation.

quite low, generally 5–6 yr for the cod and 4–5 yr for the haddock stocks. Thus, if operating, density- or stock-dependent processes would be expected to produce periodicities on the order of 8–12 (mean = 10.0) yr, which is significantly lower than the periodicities observed for these stocks (mean = 15.9 yr, $t = 2.91$, $p < 0.05$) (Table 3). Direct predator–prey or competitive interactions would generate even shorter periodicities, on the order of the generation time (i.e. 4–6 yr).

Number of recruits (log-transformed) was plotted against estimated egg production that year for each cod and haddock stock (Fig. 6). There is generally no clear linear or nonlinear relationship between egg production and recruitment for these stocks. Stock reproductive output was significantly correlated with recruitment only for Scotian Shelf (4VsW) cod ($r = 0.60$, $p \leq 0.01$). This analysis, combined with the previous examination of the expected time scale of density-dependent processes in these populations, leads us to conclude that endogenous population mechanisms are unlikely to be significant factors in producing the apparent periodic fluctuations noted in these populations.

There is also clearly no justification for fitting any particular function through the data at hand (a so-called "stock–recruitment curve"). This is not to say that density-dependent mechanisms are not operating in these populations. However, past some threshold population level generally not evident in these data, density-dependent processes simply set a mean level of recruitment; fluctuations around this mean level are presumably determined by exogenous processes. Since there is no difference between examining the residuals from a line of 0 slope and examining the original data, and no understanding to be gained by such a transformation, we proceeded to examine the relationship between recruitment to these stocks and environmental processes without explicit account taken of the influence of reproductive output. The relationship between recruitment and reproductive output will be reexamined after the environmental influences on year-class success that we consider have been statistically removed.

Recruitment and Environment

We next explored the influence on year-class success of exogenous physical and biological factors: river runoff (Sutcliffe et al. 1977, 1983); salinity as an index of slope water influence (Koslow 1984); local wind and SST; predation by 0-group mackerel; and large-scale SST and atmospheric pressure patterns (Fig. 7). Inspection indicates qualitative similarity, particularly in the low-frequency trends, among several of the environmental time series and between these and the recruitment series (Fig. 4). Preliminary analyses indicated that environmental and recruitment variables are in fact generally highly correlated in the northwest Atlantic. Recruitment to each fish stock was on average significantly correlated ($p < 0.05$) with more than three physical variables, and every physical variable was similarly correlated with recruitment to more than three recruitment series. The difficulty in understanding relationships between these recruitment and environmental time series is thus opposite to that encountered in the stock–recruitment analysis: namely, that of separating relevant from

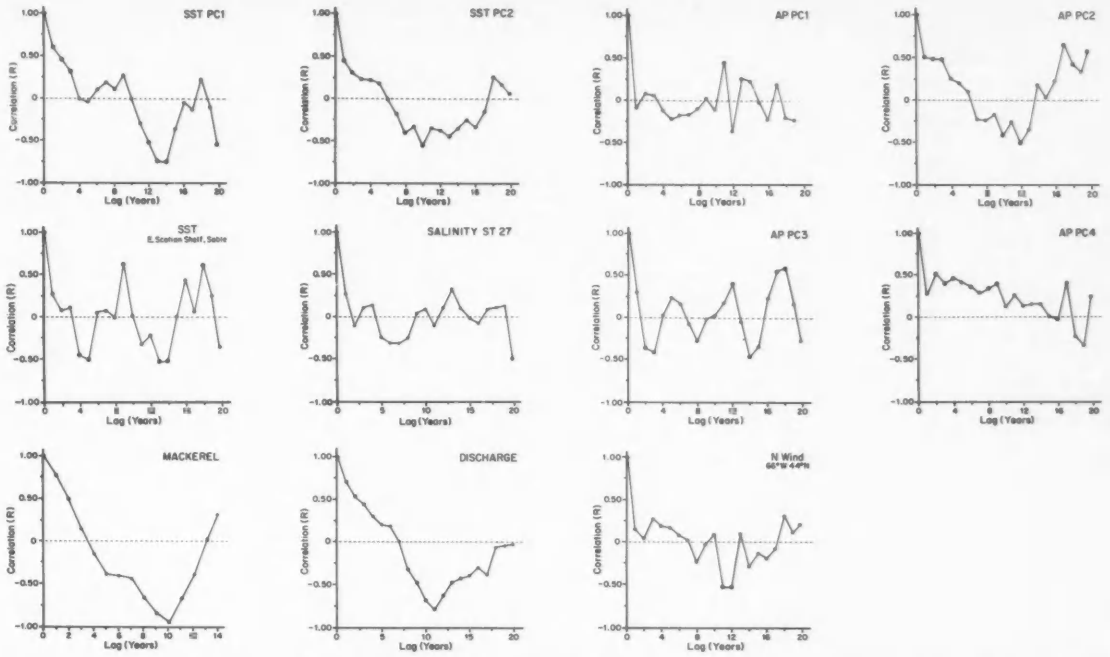

FIG. 8. Lagged autocorrelations for the environmental time series from Fig. 7. Enlarged dots indicate autocorrelations significant at $p \leq 0.10$.

spurious relationships, rather than of establishing that relationships exist. This situation arises because environmental variables in the northwest Atlantic tend to be intercorrelated (Sutcliffe et al. 1976; Koslow et al. 1986; Loucks et al. 1986). However, preliminary analysis indicated that the correlation coefficients (r) were generally less than 0.6. Under these circumstances, statistical methods, such as multiple regression analysis, can discriminate spurious relationships if relationships are linear and all relevant variables are included in the analysis. Inspection of scatterplots between recruitment and environmental variables did not reveal clearly nonlinear relationships. We proceeded by first comparing frequency-dependent characteristics of the environmental and recruitment time series. Stepwise regression analysis then provided further insight in determining which combinations of environmental variables are most clearly related to cod and haddock recruitment.

Virtually all environmental time series, except the first air pressure PC and station 27 salinity, are significantly autocorrelated at relatively long time lags, evidence of apparent periodic behavior (Fig. 8). The period length was generally between 10 and 20 yr, the time scale found for the recruitment time series, although the cycle length was somewhat too long for some variables (e.g. SST PC 1, St. Lawrence outflow, and the north component of wind off the Scotian Shelf) and too short for the local SST time series (Table 3). However, only the time series of a few of the large-scale environmental variables (e.g. SST PC 1 and 2, air pressure PC 2, and St. Lawrence River outflow) and of mackerel recruitment exhibited the highly autocorrelated, smooth autocorrelation functions generally found for the recruitment series. This suggests either that biological processes are smoothing some of the local environmental variability or that recruitment is responding to large-scale environmental processes, most of which are not adequately represented by our local variables. The latter alternative is supported by the degree of corrrelation among the recruitment series over large spatial scales. However, at this point, the autocorrelation analysis does not permit us to exclude any of the environmental variables from being considered, although several, such as air pressure PC 1 and salinity at station 27, appear less likely to be relevant to gadoid recruitment in the region.

Results of stepwise regression analysis are generally consistent with those of the autocorrelation analysis. Variables representing large-scale environmental processes (e.g. SST PC 2 and air pressure PC 2) most consistently entered the regressions with recruitment, along with local variables (e.g. St. Lawrence discharge, local winds, and mackerel predation), which are either coherent or influence processes over large spatial scales and show evidence of low-frequency periodicity. Local variables, such as salinity and SST, as well as large-scale variables (e.g. air pressure PC 1,3) that showed more high-frequency variability, were poorly represented in the regressions.

Year-class success in the two species seems to be influenced by distinct, though possibly correlated, oceanographic processes. Air pressure PC 2 and local wind variables entered the regressions for every cod stock (Table 4). Salinity and river outflow showed little significant relationship to cod recruitment, contrary to hypotheses proposed by Sutcliffe et al. (1983) and Koslow (1984) concerning the role of either freshwater discharge or salinity in the nutrient dynamics of the region. However, it is unfortunate that no time series are available of salinity on the Scotian Shelf to test these hypotheses more directly. SST generally failed to enter the regressions as well. Recruitment to the two cod stocks in the Labrador Sea is associated with a low pressure center in the northwest Atlantic,

TABLE 4. Results of regression analysis: cod recruitment (log-transformed) with annual means of SST (PC 1, 2, and local), geostrophic wind and air pressure PC 1–4, salinity, and St. Lawrence River runoff. β terms and significance indicated. ? = $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Variables entered if $p \leq 0.10$ and removed if $p > 0.10$.

		SST			Air pressure						
Stock	R^2	PC 1	PC 2	Local	PC 1	PC 2	PC 3	PC 4	Local wind	Salinity	River
Cod 1	0.32*					−0.56*					X
2J3KL	0.50**					−0.50*				0.36?	
3NO	0.67***								N 0.32*		−0.59***
3Ps	0.39**	−0.39*							NNW 0.35?		
4VsW	0.74***				0.29*	−0.28*			N 0.64***	0.23?	

TABLE 5. Regression analysis with haddock recruitment (log-transformed) with annual means of log-transformed mackerel recruitment, SST (PC 1, 2, and local), geostrophic wind and air pressure PC 1–4, salinity, and St. Lawrence River runoff. β terms and significance indicated. ? = $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. Variables entered if $p \leq 0.10$ and removed if $p > 0.10$. Regression performed stepwise forward and with backward elimination. To avoid loss of df, mackerel and Boston salinity were removed from the analysis if they did not initially enter the regressions significantly.

Haddock			SST			Air pressure						
stock	R^2	Mackerel	PC 1	PC 2	Local	PC 1	PC 2	PC 3	PC 4	Local wind	Salinity	River
						Forward stepwise						
4VW	0.77***	−0.62***					−0.40**					
4X	0.48**			−0.52*	0.50?							−0.65*
5Z	0.26*	−0.51*										
5Y	0.28*	−0.52*										
						Backwards elimination stepwise						
4VW	0.77***	−0.62***					−0.40**					
4X	0.47*			−0.45*			−0.60*			SE 0.45?		
5Z	0.56***		0.40*	−0.50**		−0.28?				NNE 0.30?		−0.46*
5Y	0.50*			−0.41?	0.63*	−0.45?						−0.74*

which appears related to northeasterly/southwesterly airflow over the region. This general wind direction and its relation with year-class success is similar to that found for Grand Banks and Scotian Shelf cod, whose recruitment is most closely associated with local offshore winds. The ecological significance of these wind/air pressure relationships are unclear, since alongshore rather than offshore winds seem to be most strongly associated with regional sea level, upwelling, and both alongshore transport and onshore–offshore exchange processes (Wright et al. 1986; Thompson 1986). The direction of the implied wind correlation is in fact opposite to that proposed by Chase (1955) for haddock on Georges Bank (which, however, has failed to be substantiated by more recent data (Grosslein and Hennemuth 1973)). However, onshore–offshore winds are the wind direction most effectively correlated with St. Lawrence discharge (Koslow et al. 1986). These correlations may therefore be indicative of the influence of prevailing meteorology on precipitation and freshwater runoff and thence on productivity of the Labrador Shelf and Grand Banks as hypothesized by Sutcliffe et al. (1983). Although the sign of the correlations is consistent with this hypothesis, testing it will require time series of precipitation or runoff from the Hudson Bay watershed. At this time, the mechanisms underlying these regressions are not clear, but the analyses were generally robust (i.e. they were unchanged whether performed stepwise forward or backward, except for west Greenland cod, for which the stepwise forward regression was clearly superior, i.e. explained a greater proportion of the variance with a single variable).

A very clear mechanism, predation by 0-group mackerel, appeared to underlie variability in recruitment to haddock stocks. When the regression analysis was performed stepwise forward, a negative relationship with 0-group mackerel emerged as either the only or the most important variable to enter three of the four regressions (Table 5).

However, the regressions with haddock recruitment were sensitive to method. When the regressions were performed stepwise backward, a range of environmental variables, including SST, air pressure, and freshwater runoff, appeared most closely related to haddock year-class success (Table 5). The generally stronger and more consistent association of haddock recruitment with principal components of SST and air pressure, rather than local SST and wind conditions, indicates a response to large-scale environmental conditions that are at present poorly understood and poorly represented by local oceanographic measurements. The sign of the relationship with St. Lawrence runoff (−) is consistent with a food chain based relationship, since increased runoff would tend to increase water column stability, thereby inhibiting mixing of nutrients into the upper water column and exchange of nutrient-rich slope water onto the shelf. However, just as Koslow et al. (1985) found that correlations of St. Lawrence discharge with SST on the lower Scotian Shelf and in the Gulf of Maine were at lags too short to be indicative of a causal relationship, the association of St. Lawrence discharge with recruitment must also be viewed with caution. St. Lawrence discharge during the first year of life entered the regressions more consistently than when lagged back 1 yr, although the outflow requires approx-

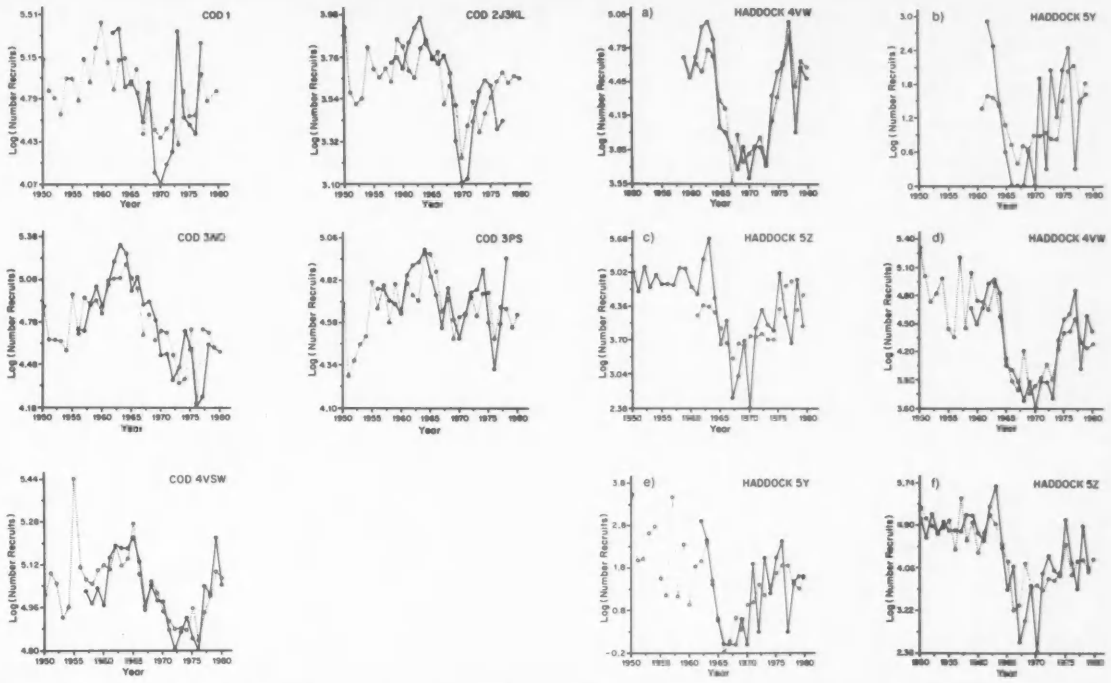

FIG. 9. Time series of predicted (○) and observed (●) recruitment to northwest Atlantic cod and haddock stocks based upon regression analyses. The variables in the regression equations and variance explained are given in Tables 4 and 5. Haddock regressions (a, b, and c) were conducted stepwise forward with mackerel entered; haddock regressions (d, e, and f) were conducted stepwise backward; and mackerel did not enter.

imately 9–12 mo to reach the area from southwest Nova Scotia to the Gulf of Maine, where the freshwater appeared to influence haddock recruitment. The relationship may therefore be based upon some further factor that is correlated with runoff. We also note that the outflow of freshwater down the Scotian Shelf tends to be concentrated nearshore, somewhat remote from the banks at the edge of the shelf, where the gadoids in this region spawn and are found during their early life history (O'Boyle et al. 1984; Koslow et al. 1985; Smith and Morse 1985). A nutrient-based food chain hypothesis for the regulation of haddock recruitment is also weakened by the weak association of salinity with haddock recruitment and its failure to enter any of the regressions. Salinity is closely linked with the contribution of slope water to the shelf water, the major source of nutrients on the shelf (Fournier et al. 1977; Houghton et al. 1978). Salinity is closely correlated with nutrient concentration on both the Labrador and Scotian shelves (Sutcliffe et al. 1983; J. A. Koslow and R. O. Fournier, unpubl. data).

Predicted and observed year-class sizes are generally in good agreement (Fig. 9). However, there is a tendency for the regressions to underpredict the best year-classes and overpredict the worst year-classes. We reopened the question of a possible stock effect, since some of the strongest year-classes were produced in the early 1960s prior to the period of heavy fishing by distant-water trawling fleets and some of the poorest year-classes at the end of the decade when spawning stocks had been reduced. However, analysis of residuals from the regressions with reproductive ouptut for the stocks revealed no significant relationships or consistent patterns. Thus, even with the influence of environment removed, the influence of stock size on recruitment cannot be detected in these time series. Some further factors or nonlinearities seem to be implicated in the formation of exceptionally good and poor year-classes.

Discussion

Although it is tempting to ascribe a large role in the decline of northwest Atlantic gadoid stocks during the mid-1960s to the intense fishing pressure being exerted by distant-water trawling fleets, there is in fact no apparent relation between stock reproductive effort and year-class success. Recruitment to all these stocks began to improve well prior to imposition of a 200-mile limit in these waters and stricter management in the mid-1970s. The rapid collapse of northwest Atlantic cod and haddock stocks during the 1960s appears to be due to the coincidence of intensive fishing with a period when the environment was unfavorable for recruitment. In the northeast Atlantic, where conditions in the 1960s were conducive to gadoid recruitment, these stocks markedly increased despite intensive fishing (Cushing 1984).

Our analysis highlights the existence of coherent, low-frequency, apparently periodic patterns of year-class success from approximately 1960 to 1980 for most cod and haddock stocks inhabiting the northwest Atlantic continental shelf. Large-scale patterns of SST and atmospheric pressure, as well as freshwater runoff, offshore winds, and the abundance of predatory 0-group mackerel, display patterns of variability qualitatively similar to the fishery recruitment series. Distinct sets of these variables are consistently associated with year-class success in each of these series. The degree of variance

explained by regression analyses (32–74% with most regressions explaining around 50%) is probably as much as can be reasonably expected, considering the error inherent in the fishery data.

However, the instability of the regression analysis with haddock recruitment highlights the limitations of statistical methods when many of the processes are intercorrelated and when the local processes presumably directly responsible for year-class success are represented in the analyses only indirectly by variables indicative of large-scale processes (e.g. the PCs). Under these circumstances, the analysis is not able to differentiate clearly among competing hypotheses. However, the high degree of correlation in year-class success among northwest Atlantic cod and haddock stocks, which is not generally found in other parts of the world (Garrod and Colebrook 1978), no doubt results from so many dominant meteorological, oceanographic, and biological processes in the region being interrelated and fluctuating more or less coherently.

A number of hypotheses have been advanced to account for the variability of cod and haddock recruitment in the northwest Atlantic (e.g. the influence of freshwater runoff (Sutcliffe et al. 1976, 1977, 1983), salinity (Koslow 1984), wind (Chase 1955)). Each was "tested" through correlations with the appropriate variable. Clearly, it is possible to be misled on the basis of simple correlations that a particular variable underlies virtually any other in the region due to widespread intercorrelations. Our multivariate analysis indicates that none of these mechanisms is predominantly responsible for year-class success in these fish populations. Furthermore, due to the nature of available data, statistical analyses in general are unlikely to resolve the bases of recruitment variability in the northwest Atlantic, although interesting hypotheses may emerge from exploratory data analysis, such as the possible influence of mackerel predation.

The evidence presented here of large-scale, apparently periodic behavior in the meteorology and physical and biological oceanography of the northwest Atlantic clearly points to the need for improved understanding of regional climatic processes and their interactions. Although climate in the northwest Atlantic appears to vary less dramatically than in some other parts of the world ocean (e.g. areas of the Pacific influenced by the southern oscillation), our study indicates that these fluctuations may nevertheless largely regulate recruitment to the region's major groundfisheries. However, it seems likely that further advances in our understanding of recruitment processes will require detailed field study to determine the actual physical and biological mechanisms underlying apparent statistical relationships.

Acknowledgements

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Competition for Spawning Space Between Brown (*Salmo trutta*) and Rainbow Trout (*S. gairdneri*) in a Lake Inlet Tributary, New Zealand

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Hayes, J. W. 1987. Competition for spawning space between brown (*Salmo trutta*) and rainbow trout (*S. gairdneri*) in a lake inlet tributary, New Zealand. Can. J. Fish. Aquat. Sci. 44: 40–47.

Effect of interference competition for spawning space on spawning success of brown (*Salmo trutta*) and rainbow trout (*S. gairdneri*) was studied in the main spawning tributary of Lake Alexandrina, New Zealand. Competition was mediated through redd superimposition and severely limited the spawning success of both species. Overall spawning success, from egg deposition to fry emergence, was 2.1% for rainbow trout and 0.2% for brown trout and was dependent on time of spawning. Brown trout spawned from April to June and rainbow trout spawned from April to October. Brown trout and early spawning rainbow trout experienced poor spawning success due to severe redd superimposition by later spawning rainbows. Late spawning rainbows experienced highest spawning success. Redd superimposition by rainbow trout caused a 94% reduction in spawning success of brown trout in an experimental section of stream. Severe intraspecific competition for spawning space, through redd superimposition, determined pattern and timing of peak rainbow fry emergence.

L'effet de la concurrence par interférence pour les aires de fraie sur le succès de la fraie de la truite brune (*Salmo trutta*) et de la truite arc-en-ciel (*S. gairdneri*), a été étudié dans le principal affluent du lac Alexandrina, Nouvelle-Zélande, où la truite va frayer. La concurrence prend la forme d'une superposition des sillons et elle nuit considérablement au succès de la fraie des deux espèces. Le succès d'ensemble de la fraie, depuis la ponte jusqu'à l'émergence du fretin, était de 2,1 % dans le cas de la truite arc-en-ciel et de 0,2 % dans celui de la truite brune; il dépend du moment de la ponte. La truite brune fraye entre avril et juin, la truite arc-en-ciel entre avril et octobre. La truite brune et la truite arc-en-ciel hâtive ont une mauvaise fraie à cause de la gravité de la superposition des sillons creusés par les truites arc-en-ciel qui viennent se reproduire plus tard. Les truites arc-en-ciel tardives ont le meilleur succès de fraie. Dans un bief expérimental d'un cours d'eau, cette superposition a réduit de 94 % le succès de la fraie de la truite brune. Une intense concurrence intraspécifique pour l'espace de fraie, vai la superposition des sillons, est à l'origine de la synchronisation et du mode d'émergence observés en période de pointe du fretin de la truite arc-en-ciel.

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The past 6 yr have seen a rapid growth in studies demonstrating interspecific competition. However, reviews by both Connell (1983) and Schoener (1983) indicate that the evidence for competition in freshwater systems is sparse, particularly for freshwater fish (but see Sale (1979), Werner (1979), and Baltz et al. (1982) for additional evidence).

Situations in which interspecific competition might be expected to be most severe and apparent arise when closely related but geographically isolated species are brought into sympatry by man. Such species may have considerable niche similarity but might not necessarily have mechanisms to alleviate interspecific competition as they might with competitors in their native ranges. Two such species are brown trout (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) which now commonly occur in mixed populations in North America, Europe, and Australasia (MacCrimmon and Marshall 1968; MacCrimmon 1971). Despite the ecological interest of these atypical mixed populations, competition between brown and rainbow trout is poorly documented. This is particularly surprising in New Zealand considering that these fish provide the basis for internationally renowned recreational fisheries. Brown trout (originally of English stock) and rainbow trout were introduced into New Zealand in the late 1800s, from Tasmania and California, respectively (MacCrimmon and Marshall 1968; MacCrimmon 1971; Scott et al. 1978). Mixed populations of these fish occur in many lakes and rivers in New Zealand and either species can dominate.

In some New Zealand freshwater systems, in which brown and rainbow trout occur together and in which rainbow trout are numerically dominant, rainbows might gain dominance through interspecific interference competition for spawning space. Later spawning by rainbow trout gives them the potential to limit brown trout populations by dislodging and destroying their eggs through redd superimposition. Larkin (1956) postulated that although competition for spawning space between freshwater fish probably occurs much less frequently than competition for food, its effects are liable to be consid-

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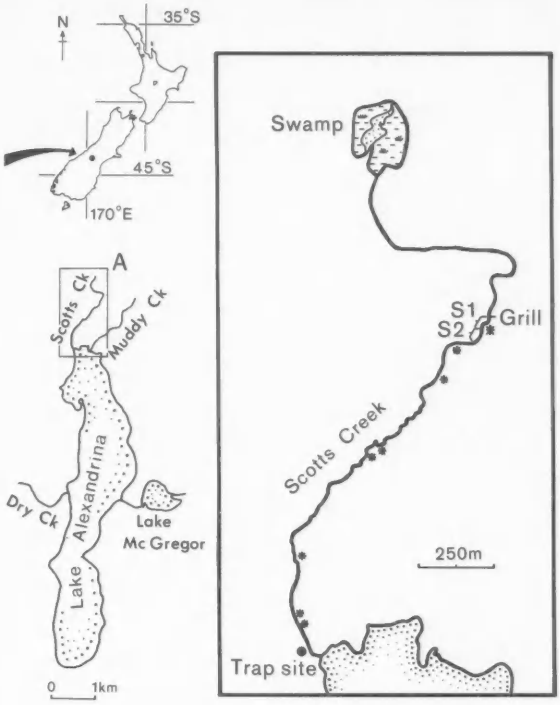

FIG. 1. Scotts Creek study area and Lake Alexandrina, with its location in the South Island, New Zealand. S1 and S2 are experimental spawning sections; asterisks indicate regularly observed redd sites.

erably more damaging to a population.

Mortality of embryos due to redd superimposition has been discussed by a number of workers (e.g. Hobbs 1940, 1948; Helle et al. 1964; Lister and Walker 1966; Hartman and Galbraith 1970; Schroder 1973). However, except for a study by McNeil (1962) on pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) spawning in three coastal Alaskan streams, very little quantitative work has been published to indicate the magnitude of such losses.

A mixed population of brown and rainbow trout dominated by the latter occurs in Lake Alexandrina, New Zealand. The spawning areas in this system are limited and are utilized by large numbers of trout. Interspecific competition for spawning space, mediated through redd superimposition, was investigated in Scotts Creek, the main spawning tributary of Lake Alexandrina, as a possible factor causing dominance of rainbow trout in this system.

Study Area

Lake Alexandrina (43°56′ S, 170°27′ E) is located in the Mackenzie Basin of the South Island of New Zealand (Fig. 1). Two inlet streams, Scotts Creek and Muddy Creek, and an outlet stream draining from Lake Alexandrina into Lake McGregor are used for spawning by brown and rainbow trout and by a small population of landlocked chinook salmon (*Oncorhynchus tshawytscha*).

Scotts Creek emerges from a small swamp approximately 1.5 km north of Lake Alexandrina and meanders for most of its length through improved pasture and modified tussock grasslands and wetlands. It is confined by well-defined banks which are high in places and frequently overhanging. Tussock and introduced grasses provide good riparian cover for most of the stream's length. The stream has a total length of 2.4 km, a mean gradient of 1.3%, and a mean discharge of approximately 0.125 $m^3 \cdot s^{-1}$ which is stable throughout the year. The habitat consists of riffle/run sequences interspersed with a few pools. The bed is composed of varying amounts of mud, sand, and rounded gravels and stones.

Mean monthly water temperatures recorded over the spawning and incubation period from May to December 1980 inclusive were 8.5, 6.9, 7.2, 7.8, 8.9, 10.3, 10.7, and 11.9°C. Maximum summer water temperatures rarely exceeded 18°C and minimum winter temperatures rarely fell below 5°C.

Spawning trout usually have access to the first 1.5 km of stream above the lake. The stream is largely unsuitable for spawning further upstream and access to this area is usually prevented by a removable grill which is dropped into position at the start of each spawning season.

During the study approximately 940 m of the stream was utilized for spawning. The extent of the spawning bed was described in terms of length rather than area because the stream was too narrow for more than one discrete redd across its width. There was sufficient space for approximately 337 females to spawn concurrently. This figure represents the maximum number of redd sites available (Hayes 1984). Of these sites, 24 were on "coarse" substrate (geometric mean particle diameter (Shirazi and Seim 1979) d_g = 23.8 mm, variance, σ_g = 2.35), 147 were on "fine" substrate (d_g = 3.5, σ_g = 2.6), and 166 were on "medium" substrate (d_g = 5.7, σ_g = 3.3) (Hayes 1984).

Materials and Methods

Information on size and timing of the spawning runs of brown and rainbow trout in Scotts Creek was obtained from daily totals of fish trapped at the stream mouth. The trap was operated continuously from 5 May to 15 October 1980 and from 9 April to 18 June 1981. Subsequently, during 1981, it was operated for periods of 1 wk at approximately monthly intervals until October to establish the seasonal timing of rainbow trout spawning.

Fecundity, defined here as the number of ripening eggs in a female prior to spawning, was determined for brown and rainbow trout from samples of 17 and 28 females, respectively, collected from the fish trap during the 1980 and 1981 spawning seasons.

Fence traps of the design used by Armstrong and Argue (1977) were used to trap juvenile downstream migrants. Outmigrant 0+ fish were trapped near the stream mouth continuously from the onset of emergence in early August 1980 to 18 June 1981. The following spring, the trap was operated intermittently to establish the approximate pattern and timing of fry emergence.

Population estimates of newly emerged fry remaining in the stream were made by extrapolating population density estimated in three 50- to 60-m representative sections to total area of the stream. Estimates of populations within sections were made by the removal method (Zippin 1958) using a generator-powered, pulsed DC fish shocker.

Potential egg deposition was compared with the observed number of emergent fry to calculate overall spawning success (from egg deposition to emergence) for the two species.

Relationship Between Time of Spawning and Spawning Success

To determine whether spawning success was dependent on time of spawning, I compared the relative spawning success of rainbow trout whose progeny emerged during three selected portions of the emergence period, before, during, and after peak emergence. The periods of egg deposition from which the selected groups of fry originated were determined by back-calculating incubation times from fertilization to emergence. It was assumed that numbers of emergent fry (<30 mm) caught in the fry trap were proportional to numbers of fry emerging in the stream. It was known that approximately 90% of rainbow fry migrated to the lake immediately following emergence (Hayes 1984). Furthermore, the density of fry in the stream prior to early November was very low (<0.6 fry·m^{-2}), indicating that the number of early emerging fry was not significantly underestimated by a lower tendency of these fry to emigrate.

Incubation time from fertilization to hatching was calculated using

$$\log D = b \log (T-\alpha) + \log a$$

where D is days from fertilization to 50% hatch, T is mean water temperature, α is a temperature correction factor, and b and a are species-specific constants (Crisp 1981). Incubation time from fertilization to emergence was estimated by multiplying the length of the period from fertilization to hatching (in days) by 1.714; this factor is the ratio of the former over the latter at 11°C, the only complete data from fertilization to emergence available in this study. A correction factor of 15 d was added to account for an apparent underestimation by the method (Hayes 1984). Mean water temperatures used for the above calculations were obtained by averaging mean daily temperatures for each month, working backwards from time of emergence to the probable month of fertilization. The numbers of females arriving on the spawning ground during each of the selected spawning periods were used to calculate egg deposition during each period. It was assumed that number of arrivals into the stream approximated number of spawners. A gross comparative measure of spawning success was then calculated for fish spawning in each period, using the estimated number of eggs deposited and the number of emergent fry trapped during each corresponding section of the emergence period.

Redd Superimposition

The frequency with which redds were superimposed during the 1980 spawning season was estimated from maps of successive redds recorded on eight initial redd sites positioned at approximately regular intervals over the length of the stream (Fig. 1). At each site, four stakes were arranged in a rectangle enclosing the initial redd and driven into the banks. Then, along each bank a nylon line was suspended between the stakes. The position and dimensions of the initial redd at each site were recorded by measuring the distance from the perimeter of the redd to the lines on the banks at 10-cm intervals using a length of nylon monofilament as a "straightedge." The sites were observed regularly over the length of the estimated incubation period of the initial redd. All subsequent redds at each of the sites were recorded and mapped. The area of each successive redd superimposition was calculated and expressed as a percentage of the total area of each redd superimposed.

During the 1981 spawning season, an experiment was carried out to estimate mortality of brown trout eggs and alevins from redd superimposition by later spawning rainbow trout. On 26 April, five female and four male brown trout from the spawning run were placed in each of two contiguous 50-m sections (1 and 2) of Scotts Creek (Fig. 1). The fish were confined within each section by grills placed at both ends until they had completed spawning. The redds in the lower Section 2 were then exposed to superimposition by later spawning rainbow trout. Ranges of fork lengths of fish released into Sections 1 and 2, respectively, were 53.0–62.0 and 49.5–62.0 cm; ranges of weights were 2.4–3.5 and 2.0–3.9 kg. Total fecundity of each group was estimated by summation of individual fecundities calculated from the length fecundity relationship for brown trout. To calculate potential egg deposition from total fecundity, I allowed for a 2.5% loss due to egg retention within females, nonfertilization, and nonlodgement of eggs in the substrate (Hobbs 1948). Spawning substrate in both sections was composed entirely of "medium" size gravel. I allowed for an incubation period (from time of fertilization to time of emergence) of the brown trout eggs of approximately 11 wk. At the end of this period, after removing all adults present in Section 2, I installed fry traps at the downstream ends of both sections to catch all downstream migrating fry. These traps were operated continuously until emergence was judged to be complete. At this time the remaining brown trout fry in each section were estimated by electrofishing using the removal method (Zippin 1958).

Spawning success, the quotient of the number of free-swimming fry divided by the estimated number of eggs deposited, of the two groups of brown trout was compared. These were minimum estimates because the mortality of free-swimming fry was not accounted for. The mortality of eggs and alevins due to redd superimposition (M) was calculated.

$$M = \frac{P-O}{P}$$

where P and O are the potential and the observed number of free-swimming fry, respectively.

Results

Spawning Migrations

Observations and trapping records showed that spawning in Scotts Creek began in early April for both species and continued to July for brown trout and to the middle of October for rainbow trout (Fig. 2). The adult fish trap was installed on 9 May 1980, by which time 91 rainbow trout (59 males and 32 females) and 22 brown trout (9 males and 13 females) were already present in the stream. Previous observations on numbers of spawners and redds indicated that few fish would have spawned and returned to the lake before the trap was installed. In 1980, 2932 spawners were trapped entering the stream, mostly rainbow trout (Table 1).

During the 1981 spawning season when the trap was operated continuously from 9 April to 18 June, 512 rainbow trout, 116 brown trout, and 34 chinook salmon entered the stream to spawn. This was 1.6, 1.9, and 3.4 times as many, respectively, than was recorded over the same period the previous season. However, the pattern of the rainbow trout run was similar in the two seasons, with a pronounced peak late in each season (Fig. 2). This pattern is considered typical of the spawning runs currently occurring in Scotts Creek. Casual observations of the

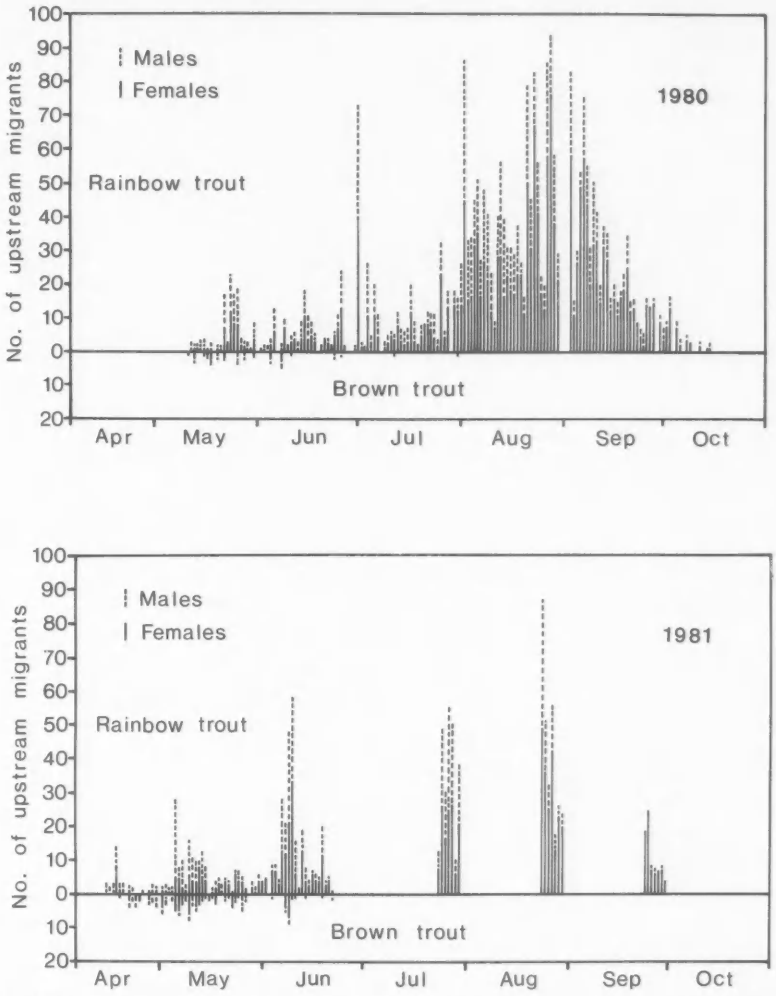

FIG. 2. Daily number of upstream migrant brown and rainbow trout trapped entering Scotts Creek in 1980 and 1981.

spawning runs made over a number of years by South Canterbury Acclimatization Society field staff support this view.

Mean fork lengths and weights of spawners were similar between the two species and between years (Table 1).

Fecundity

Egg number and fish length were significantly correlated in female brown trout ($P < 0.01$, $r = 0.851$), but not in female rainbow trout ($r = 0.229$). For brown trout the relationship between fecundity (F) and length (L) is given by the G.M. functional regression (Ricker 1973):

$$\ln F = 2.823 \ln L - 2.790.$$

For rainbow trout, fecundity was estimated by the mean number of eggs per female which was 5272 ± 416.

Frequency of Redd Superimposition

Frequency of redd superimposition was high throughout the spawning season. Approximately 80–90 sites (mostly coarse and intermediate substrate categories) appeared greatly favoured by fish. These were situated at the heads of riffles or in runs where the bed sloped upward in the direction of the flow. Such sites were used repeatedly through the spawning season and during peak spawning were occupied continuously. At one observation site, eight redds were constructed during one incubation period (from initial egg deposition to emergence). Of 46 redds observed for more than 1 mo, 89% were partially or wholly redug by subsequent spawners. The mean proportion of redd area superimposed was approximately 65%, and mean frequency at which redds were superimposed was once every 19 ± 4 d.

Digging activity on some sites was so intense that it eventually resulted in displacement of most of the gravel downstream, exposing underlying boulders, and rendering the site unsuitable for further spawning. Observations made over three spawning seasons indicated that such intense digging activity

TABLE 1. Species, numbers, and mean lengths and weights (±SE) of upstream migrants passing through the adult fish trap in 1980.

		No.	Species totals	Percent of total migrants	Mean length (cm)	Mean weight (kg)
Rainbow trout	♂	997	2860	97.5	56±0.7	2.3±0.02
	♀	1863			56±0.6	2.5±0.01
Brown trout	♂	20	62	2.1	55±1.4	2.4±0.14
	♀	42			55±0.8	2.8±0.16
Chinook salmon	♂	3	10	0.3		
	♀	7				

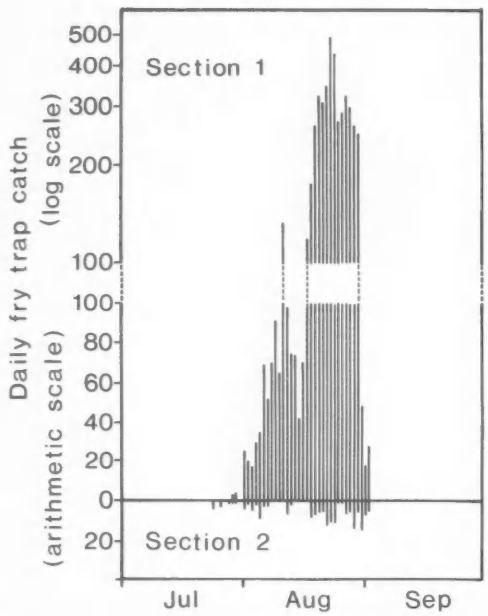

FIG. 3. Daily catches of brown trout fry caught in fry traps below each of the experimental sections (1 and 2) over the period 23 July to 2 September.

was common and caused continual changes in streambed topography.

Once a redd had been dug, there appeared to be a greater likelihood of subsequent redds being made in that location. That is, redds appeared to be contagiously distributed. This was particularly obvious late in the season with previously unused sites being intensively utilized once an initial redd was dug.

Redd superimposition would be expected to increase as spawner density increased. Although general observations supported this view, results from the regularly observed redd sites did not. There was no obvious trend in frequency of redd superimposition on any site. However, since site attractiveness continually changed with digging activity of fish, the frequency of redd superimposition at any one site need not necessarily exhibit a general trend.

Time of redd site occupation was variable, ranging from approximately 1 to 4 d, with the mode at about 3 d.

TABLE 2. Spawning success of female brown and rainbow trout in each of the experimental sections (approximate 95% confidence limits).

	Section 1 (control)	Section 2 (experimental)
Total fecundity (number of eggs)	27 246±9038	26 796±8939
Potential number of eggs deposited	26 565±8812	26 126±8716
Number free-swimming fry	5911±151	358±37
Spawning success	22.3±7.7	1.4±0.4

Mortality due to Redd Superimposition in Experimental Sections

All of the brown trout that were released into the experimental sections had completed spawning within 8 d, and each female constructed a single redd (a single linear array of nests).

Subsequently, rainbow spawner densities in Section 2 were high and redd superimposition was severe. Large congregations totalling over 50 fish were observed prior to installation of the fry traps. The original brown trout redds became unrecognizable because streambed topography had been altered considerably by digging activity of the rainbow trout.

There was a marked contrast in numbers of brown fry produced in each section. Total numbers of brown fry captured in each section were 5911 and 358 in Sections 1 and 2, respectively. From 23 July to 2 September, 5213 and 169 brown fry were trapped in Sections 1 and 2, respectively, and peak fry emergence occurred between 18 and 30 August (Fig. 3). Emergence was judged to be finished by 2 September. The population of brown fry remaining in Sections 1 and 2 at that time was 698 and 189, respectively.

Spawning success was much lower in Section 2 than in Section 1 (Table 2). In the absence of redd superimposition there was 77.7% mortality of eggs from fertilization to emergence. The estimated loss of eggs and alevins through redd superimposition in Section 2 was 94 ± 7%.

Spawning Success

The potential population fecundities (total number of eggs for all females) of brown and rainbow trout in Scotts Creek during the 1980 spawning season were 187 340 ± 77 027 and 9 753 200 ± 769 600 eggs, respectively (95% confidence limits).

Allowing for 2.5% loss of eggs, I estimated potential depo-

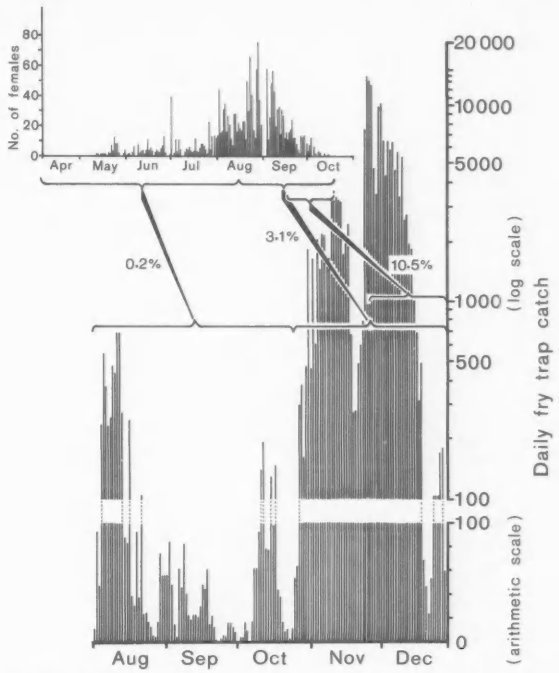

FIG. 4. Comparative success of female rainbow trout arriving on the spawning ground during three periods of the 1980 spawning season, showing numbers of females and corresponding numbers of their emergent fry.

sitions of 182 657 ± 75 102 and 9 509 370 ± 750 360 eggs for brown and rainbow trout, respectively. Total fry output from the redds in 1980 was estimated for each species by combining resident population estimates determined as close to the end of emergence as possible, with the number of 0+ fish caught in the fry trap up until that time. These were minimum estimates because mortalities of migrant and resident fry were not accounted for. For rainbow trout, emergence was judged to be finished by about 1 January 1981. The resident 0+ rainbow trout population on 1 January was approximately 20 000 fish. By this time, 186 260 rainbow trout fry had been caught in the trap; combining this figure with the resident population estimate gave a minimum estimated output of 206 260 rainbow fry for the 1980 season. Brown trout fry emergence was judged to be finished by about 12 September 1980. The earliest population estimate made was on 13 November 1980, which was used as an approximation for the population of 0+ trout at the end of emergence. Approximately 319 age 0+ brown trout were in the stream on 13 November, and 120 had been caught in the fry trap by that time. Summation of these totals gave a minimum estimate for brown trout fry output for the 1980 season of 439 fry.

From the estimates of egg deposition and resulting fry output, I calculated that overall spawning success of brown and rainbow trout in 1980 was approximately 0.2 ± 0.17 and 2.1 ± 0.1%, respectively.

Comparison of the relative spawning success of rainbow trout whose progeny emerged before, during, and after peak emergence showed that spawning success was dependent on time of spawning. Late rainbow spawners had higher spawning success than early spawners; spawning success increased from 0.2 to 10.5% (Fig. 4). The most successful fish spawned sometime after 14 September.

Discussion

In comparison with other New Zealand waters supporting cooccurring populations of brown and rainbow trout, Scotts Creek probably represents an extreme example of interference competition for spawning space between these species. Study of this system reveals the potential severity of competition between brown and rainbow trout, and more generally, the effectiveness of interference competition for spawning space in limiting the abundance of a competitor. Severe interference competition for spawning space, mediated through destruction of eggs by redd superimposition, occurred between these species, and also within the rainbow trout population, favouring late spawners. Size and timing of the spawning runs determined the intensity of competition for spawning space, and which species was favoured.

"In general, interference by individuals in a population will cost them something in terms of rate of resource exploitation and therefore rate of population growth, since they will have to spend some time and energy in acts of interference which consume time and energy that would otherwise be devoted to resource exploitation" (Case and Gilpin 1974). While this generalization is true for most forms of interference competition, it does not hold for competition for spawning space, mediated through redd superimposition. Such competition is "gratuitous" and acts indiscriminantly of species as a result of normal spawning activities. Although each fish gains access to the spawning bed, the sequential use of the bed means that only the progeny of the last spawners are able to successfully utilize the resource. This form of competition costs nothing but has a devastating effect on a competitor by directly reducing it's abundance.

In Scotts Creek, the small size of the spawning bed and limited number of preferred sites led to a very high frequency of redd superimposition, particularly on preferred sites, and this was aggravated further by contagious distribution of redds. McNeil (1967) demonstrated that pink salmon redds were contagiously distributed in an experimental spawning channel. Presumably, contagious distribution of redds arises because the construction of a redd favourably modifies the physical conditions for spawning on that site, thereby making it more attractive to later spawners.

Many workers have reported the occurrence of redd superimposition but few have been able to estimate losses of embryos resulting from it. Hobbs (1940, 1948) discussed the phenomenon in brown and rainbow trout waters in New Zealand and speculated that losses were fairly extensive, possibly averaging 30%. Krogius and Krokhin (1948) and Krogius (1951) attributed extremely poor spawning success to redd superimposition by later spawners when runs of sockeye salmon were large and the spawning period prolonged. McNeil (1962) described an asymptotic relationship between density of female pink and chum salmon spawners and those depositing their eggs safely, from which he estimated mortality from redd superimposition at 25% at the highest observed density of 15 spawners · 100 m^{-2}. Hartman and Galbraith (1970), reporting on the Gerrard rainbow trout spawning stock in the Lardeau River, Canada, found that approximately 26% of 217 redds were superimposed and that about 239 000 eggs were displaced

through redd superimposition and failure to lodge in the gravel after spawning.

With the experimental section of Scotts Creek, it is important to consider whether the 94% loss of embryos due to redd superimposition was an unbiased representation of that which occurred throughout the stream. It is possible that upstream migrating adults may have accumulated below the barrier in the experimental spawning section (Section 2), possibly resulting in higher frequency of redd superimposition than in the rest of the stream. Such a disparity would be greatest early in the spawning season, before the stream's capacity for spawning space was exceeded. However, losses due to dislodgement of eggs eventually would have reached maximum levels within and outside the experimental section as overall spawner density increased later in the season. Mortality due to redd superimposition in the experimental section probably represents the maximum loss that occurred in the most preferred sites.

Narrowness of the spawning bed promoted complete rather than partial redd superimposition. Thus, losses due to redd superimposition are probably higher in Scotts Creek than in wider spawning beds with similar spawner densities.

Intensive digging activity of rainbow females throughout the spawning season appeared to improve the quality of the spawning gravel by removing fine particles and organic detritus. This would have been most beneficial to the progeny of late spawners. McNeil (1962) recorded a significant decrease in percentage of fine particles in a pink and chum salmon spawning bed in association with spawning. He estimated that spawning females doubled permeability of the top 15 cm.

Analysis of spawning success versus time of spawning showed that late rainbow spawners experienced the highest spawning success. Expanding on this theme, close inspection of the pattern of rainbow fry emergence revealed that the females responsible for peak emergence spawned within a short space of time (less than 2 wk) near the end of the spawning period. Evidence for this conclusion comes from the similarity in temporal spread of the peak rainbow fry emergence (Fig. 3) with that of brown fry in Section 1 (Fig. 2), a pattern that resulted from five females spawning within 1 wk. Increasing water temperature might be expected to synchronize emergence times, thereby compressing the observed rainbow fry emergence peak. But in Scotts Creek this phenomenon could only account for a compression of the emergence peak by 11 d (or by 17%).

The late narrow peak rainbow fry emergence period can best be explained if, during peak spawning, the number of female spawners greatly exceeded the spawning space available, so producing an accumulation of spawners. During this period almost complete displacement of eggs through redd superimposition would occur (McNeil 1962) and would continue until near the end of the spawning period when declining numbers of new arrivals allowed the accumulation of spawners to be eliminated. The vast majority of eggs deposited at the time when the accumulation of spawners was cleared would escape displacement and survive to emergence because by that time there would be few subsequent spawners.

Interference competition for spawning space could cause selection for late spawning, and presumably it is one driving force in segregation of spawning time and place of different races and species of naturally cooccuring salmonids. Inheritance of time of sexual maturation has been well documented in salmonids (Leider et al. 1984). Certainly, the very skewed temporal pattern of spawning implies that selection for late spawners is occurring in Scotts Creek. On the other hand, this pattern of spawning, with a pronounced peak late in the season, may simply be a consequence of a predominantly spring spawning stock in New Zealand. However, in 1962 the Scotts Creek spawning run did not exhibit this pattern and instead was uniformly distributed (Moore et al. 1962). Observations by South Canterbury Acclimatization Society field staff indicate that the present pattern of the rainbow spawning run appears to have developed within the past 10 yr. It seems to have been associated with improvements to the spawning bed, and subsequent natural fry propagation, and the recent cessation of artificial releases of rainbows into the lake (Hayes 1984). The available evidence suggests that since Scotts Creek has become a significant natural fry producing system free from artificial perturbation, the rainbow spawning run has developed a late spawning characteristic which is consistent with a population limited by redd superimposition.

Awareness of the potential severity of redd superimposition and its influence on the relationship between pattern of spawning and pattern of fry emergence is important in management of salmonid spawning streams. Given that severe redd superimposition is occurring, spawning runs could be manipulated to maximise spawning success. For example, in Scotts Creek two batches of spawners would experience successful spawning if rainbows which currently spawn in the middle of the season (July and August) were denied access to the stream. This would allow brown trout and early spawning rainbow trout embryos to develop to emergence free from disturbance by redd superimposition, and yet would not impair spawning success of late spawners which account for virtually all of the current rainbow trout fry output. Such manipulation could potentially double the present fry output from Scotts Creek.

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Density-Dependent Growth of Age 1 English Sole (*Parophrys vetulus*) in Oregon and Washington Coastal Waters

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Peterman, R. M., and M. J. Bradford. 1987. Density-dependent growth of age 1 English sole (*Parophrys vetulus*) in Oregon and Washington coastal waters. Can. J. Fish. Aquat. Sci. 44: 48–53.

We tested whether English sole (*Parophrys vetulus*) in Oregon and Washington waters show density-dependent growth. We found that there is a significant negative effect of cohort abundance on annual growth rate of age 1 fish, but not on growth of ages 2–7. Unlike most similar studies of density dependence, this result was not confounded by time trends in abundance and growth. The multiple regression of age 1 growth on cohort abundance and temperature accounted for 91% of the interannual variation in growth, which was a significant increase in r^2 over that of the previously published relation with temperature alone. However, stock assessments which take into account only the previously published temperature effect on growth for this stock will probably not seriously overestimate the impact of management regulations which increase cohort abundance.

Nous avons vérifié si le taux de croissance de la sole anglaise (*Parophrys vetulus*) dans les eaux des États de l'Orégon et de Washington était fonction de la densité. Nous avons établi que le nombre d'individus d'une cohorte exerce un effet négatif significatif sur le taux de croissance annuel du poisson de 1 an, mais non pas sur celui du poisson de 2 à 7 ans. Contrairement à la plupart des autres études semblables sur l'influence de la densité, ce résultat n'était pas démenti par les tendances temporelles de l'abondance et de la croissance. L'effet de régression multiple de la croissance des poissons de 1 an sur le nombre d'individus de la cohorte et la température expliquait 91 % de la variation interannuelle du taux de croissance, ce qui est une augmentation importante du r^2 par rapport à celle publiée antérieurement et établissant un rapport uniquement avec la température. Toutefois, les évaluations de population publiées antérieurement et ne considérant que les effets de la température sur le taux de croissance ne surestimeront probablement pas énormément les répercussions des règlements de gestion qui visent à augmenter le nombre d'individus d'une cohorte.

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Density-dependent growth has been documented in the early life stages of a wide variety of marine and freshwater fishes. Some examples are haddock, *Melanorammus aeglefinus* (Beverton and Holt 1957), North Sea herring, *Clupea harengus harengus* (Hubold 1978; Burd 1985), sockeye salmon, *Oncorhynchus nerka* (Hyatt and Stockner 1985), and many other species reviewed by Ware (1980). During the course of empirically identifying components for a population model, we tested whether English sole (*Parophrys vetulus*) off the Oregon and Washington coast also showed density-dependent growth. Because Kreuz et al. (1982) reported a significant effect of June temperature at 100 m depth on growth of sole, we tested whether cohort abundance had a significant additional effect on growth, over and above the temperature influence. Our results indicate that such an additional effect of fish abundance on age 1 growth does exist.

Methods I

Density Dependence

We analyzed previously published data for the English sole population which occupies the portion of the Washington and Oregon coast designated Area 3A by the Pacific Marine Fisheries Commission. Because of the high proportion (95%) of females in the catch, we examined data only on females, as have other researchers working on this population (e.g. Hayman et al. 1980).

Kreuz (1978, table 2) and Kreuz et al. (1982) backcalculated annual growth increments in fish length for 1961–74 from (1) measurements on interopercular bones and (2) the relation between bone size and fish length. Growth increments were estimated only from samples taken from fish ages 5–7; younger fish were subjected to size-selective fishing (Kreuz et al. 1982), and sample sizes for older ages were too small to give reliable results. Detailed methods for calculating growth increments are given in those papers. In our analysis, we used Kreuz's (1978) annual growth rate data for ages 1–7 for the Astoria population. "Age 1 growth" is the growth between the first and second annulus. Growth data for age 0 fish were not available because the focus of the bone was often not discernible (Kreuz 1978).

English sole live on the sea bottom after undergoing metamorphosis. To explore the effect of temperature on juveniles and older fish, Kruse and Huyer (1983) developed an algorithm using sea level height data to calculate June temperature at a depth of 100 m. Kreuz et al. (1982) found this temperature to be correlated with growth of age 1 (one annulus) fish. In our analysis, we used the water temperatures generated by Kruse

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and Huyer's (1983) algorithm and tested for additional effects of stock abundance on growth.

We fit a multiple linear regression to the annual mean growth rate, G (centimetres), of each of the seven age groups available to us (1−7), using the temperature, T (degrees Celsius), at 100 m depth and the appropriate fish biomass, B (kilograms), as independent variates:

$$(1) \quad G = a + b_1T + b_2B.$$

We tested the null hypothesis that $b_2 = 0$, using a 0.05 level of significance. We used a one-tailed t-test because we expected on the basis of research on other fishes (e.g. Ware 1980) that increased abundance would decrease growth rate. For the regressions, each mean annual growth rate, by age, was weighted by its sample size, as given in Kreuz (1978). We expected that only the youngest ages would show density dependence (Ware 1980), but for completeness we examined all seven age groups for which we had growth data.

The independent variable of biomass, B, used in equation (1) differed between two groupings of age classes, 1−3 and 4−7. For each of ages 1−3, we used an index of the biomass of the cohort of that age for B, whereas the pooled biomass of all ages 4−9 was used for B in regressions for ages 4−7. This difference is due to the geographical distribution of different age groups. Laroche and Holton (1979) showed that age 0 and 1 fish were separated spatially and very few age 2 or older fish overlapped with age 1 fish. Ketchen (1956) also concluded that age 1 fish were relatively isolated (inhabiting depths <37 m), with each successive age group through age 3 being distributed farther offshore in deeper waters. Adults (ages ≥4) are mixed spatially and remain between 45 and 90 m depth (Ketchen 1956).

Based on these geographical distributions of age classes, the biomass estimates used in our seven regressions with equation (1) were calculated as follows. The adult stock biomass used in the age 4−7 multiple regressions was the sum of the age 4−9 biomasses. Age 10−13 fish were not included here because the cohort analyses for ages 4−13 would have generated too few complete cohorts to permit testing for density dependence. Inclusion of the age 10−13 fish would add only about 8% to the total biomass.

To calculate age 4−9 biomass, we used (1) the subset of numbers at age from Krue's (1983) table 4, as calculated from cohort analysis, which coincided with years in which we had annual growth rate data (e.g. age 4 growth for 1964−74), (2) average length at age (Kreuz 1978), and (3) a length−weight relation from Ellen Pikitch (Marine Science Center, Newport, OR, pers. comm.):

$$W = 1.47 \times 10^{-5} L^{2.916}$$

where W = weight (kilograms) and L = total length (centimetres).

Because recruitment to the fishery begins at age 4, abundance estimates for ages 1−3 were not available. We used the biomass of the appropriate age 4 group as a surrogate for the abundance of the cohort at earlier ages. For example, the 1970 age 4 biomass was used in the regression with growth of age 1 in 1967, with age 2 in 1968, etc. This method is based on the assumption that relative year-class strength at age 4 is established by age 1. This assumption is probably valid because empirically based simulation models of this population have shown that most of the variability in year-class strength is determined during the early life history, prior to age 1 (Kruse 1983; Peterman et al. 1987). Research on other marine fish species also supports this view (Rauck and Zijlstra 1978 for plaice, *Pleuronectes platessa*; Burd 1985 (and Wood 1983, cited therein) for North sea herring).

TABLE 1. Multiple regression statistics for equation (1), for annual growth rate (cm) as a function of June temperature at 100 m depth (°C), and stock biomass (kg). P is the probability concerned with the one-tailed test of the hypothesis that $b_2 = 0$; n = sample size (yr).

Age	n	a	b_1 (temp.)	b_2 (biomass)	P	Multiple r^2
1	11	18.93	−1.50	-2.94×10^{-7}	0.024	0.91
2	11	16.50	−1.37	8.66×10^{-8}	0.56	0.69
3	11	8.79	−0.57	1.69×10^{-8}	0.52	0.50
4	11	4.48	−0.23	2.01×10^{-7}	0.82	0.32
5	10	4.32	−0.32	2.27×10^{-7}	0.90	0.64
6	9	1.84	−0.04	1.52×10^{-7}	0.85	0.20
7	8	2.47	−0.15	1.32×10^{-7}	0.95	0.70

Results I

Density Dependence

Age 1 was the only age class of English sole in which there was a significant negative effect of abundance on growth, above the effect of temperature (Table 1; Fig. 1). The resulting multiple r^2 was 0.91 ($P < 0.001$), which was a statistically significant improvement over the $r^2 = 0.85$ accounted for by the regression of age 1 growth on temperature alone reported by Kreuz et al. (1982).

Methods II

Management Implications

We explored the management implications of the observed density dependence in age 1 growth by calculating the fate of a single representative cohort of English sole. We compared the catch biomass and number of eggs generated over the life of a cohort under two alternative assumptions: (1) that the stock dynamics were density independent, as a modeler would assume on the basis of previously published research, and (2) that stock dynamics were the same as in (1) except density-dependent growth of age 1 fish was added. For high and low abundance year classes, we quantified the error in catch and egg production forecasts that would result from not accounting for density-dependent growth. Such strong and weak year classes could arise either from natural causes or from management actions which affect the spawning stock. The difference between forecasts of the density-independent and density-dependent models was calculated as a percentage of the latter, because we assume that density-dependent growth occurs in nature.

Our calculations were made using a model which included several empirically based components common to the two assumptions noted above. Egg production was calculated from female abundance, size, maturity, and fecundity components. Survival and growth processes were used to calculate catch biomass. Growth was the only component we varied to compare the density-independent and density-dependent cases.

Egg production

The total egg production of a cohort over ages 4−13 was calculated as the product of numbers of female fish alive at

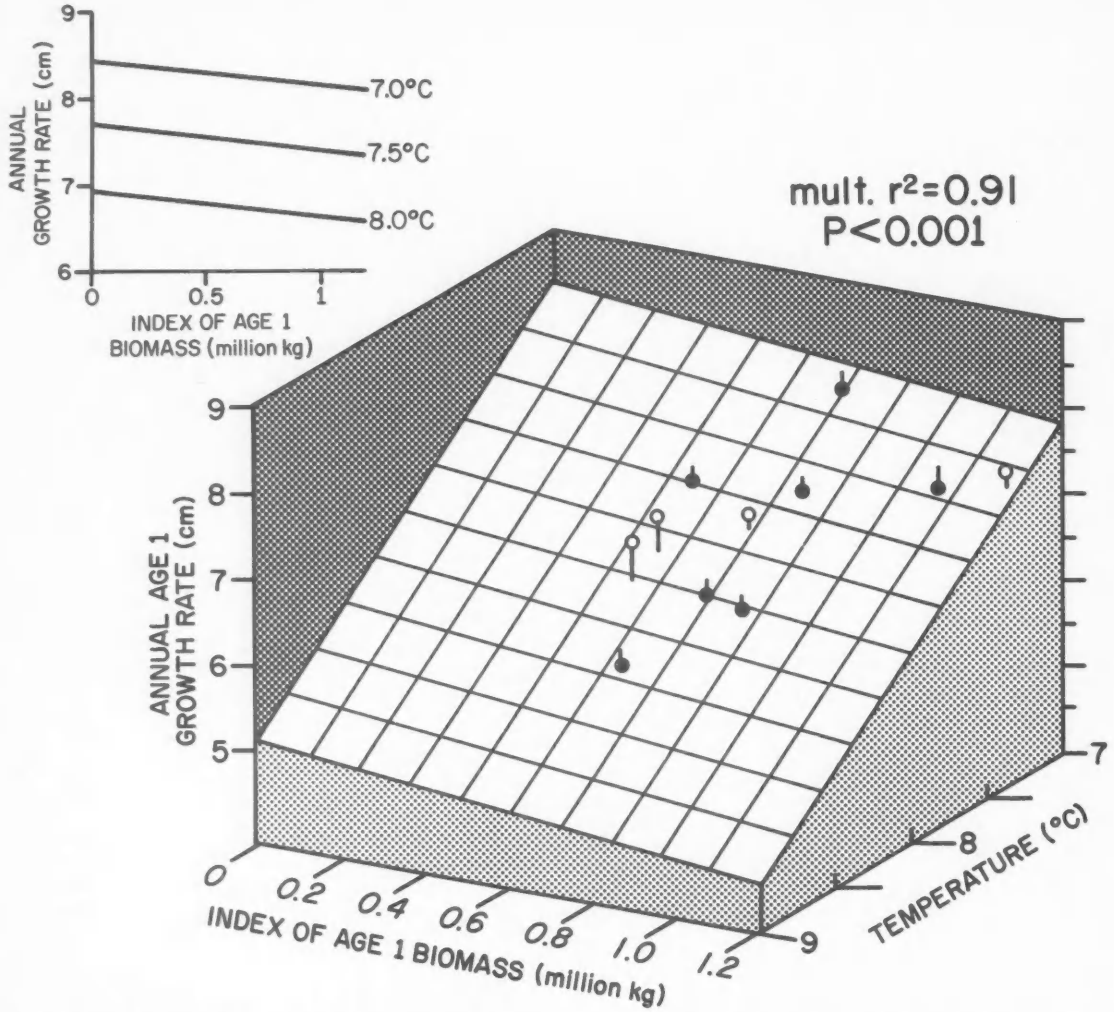

FIG. 1. Regression plane showing the relation among annual growth rate of age 1 English sole, June bottom temperature, and an index of age 1 cohort biomass (as represented by the appropriate "hindcasted" age 4 biomass; see text). Multiple $r^2 = 0.91$, $P < 0.001$; see Table 1. Open circles are observed data points which are positive residuals from the regression plane and solid circles are negative residuals. Inset shows this age 1 density-dependent growth relation as a family of three temperature functions, as described by the multiple regression equation of Table 1.

each age, the proportion of mature fish at the given lengths at age, and the fecundity. The proportion mature as a function of length was calculated from an ogive fit to Harry's (1959) maturity data. For fecundity, we used a relation fit to Harry's (1959) fecundity data by Kruse (1983):

$$E = 4.558 \times 10^{-4} L^{3.6097}$$

where E is number of eggs per female per year and L is total female length (millimetres).

Cohort abundance

Two starting abundances for the age 4 fish were used for comparison: the lowest (2.406×10^6 fish) and highest (5.724×10^6) were the extremes in age 4 abundances estimated by cohort analysis of the 1955–70 year classes (Kruse 1983). Survival of each cohort followed the standard model

$$N_{t+1} = N_t e^{-(F+M)}$$

where N_t = number of fish alive at age t, F = instantaneous fishing mortality rate, and M = instantaneous natural mortality rate. We assumed that $M = 0.294$ for all of ages 4–13 (Hayman et al. 1980), F for age 4 = 0.145, and F for ages 5–13 = 0.278 (average of 1963–72 estimates from Hayman et al. 1980).

Yield

The catch biomass generated by the cohort over its life was simply the number caught at each age times the weight at that age. The number caught was given by the Baranov catch equation (Ricker 1975):

TABLE 2. Average annual growth increment between ages t and $t + 1$ used in calculations of the implications of density-dependent growth at age 1. The asterisk indicates that the growth increment for age 1 was either 7.02 cm if density-independent growth was assumed or it was calculated from equation (1) if density-dependent growth was assumed.

Age t	Increment (cm)
1	*
2	6.10
3	4.44
4	3.19
5	2.26
6	1.95
7	1.60
8	1.03
9	0.78
10	0.58

$$C_t = (F_t A N_t)/Z$$

where $Z = F_t + M$ and $A = (1 - e^{-Z})$. We used the length–weight relation shown above to convert the lengths at a given age to weight per fish.

Length at age

At the beginning of age 1, fish were assumed to be 11.4 cm (Barss 1976). Growth between the start of age 1 and 2 was the only component which differed between the density-independent and density-dependent calculations, because no other age showed evidence of density-dependent growth (see Results I). In the density-independent case, age 1 growth was calculated only as a function of June water temperature, using the relation reported by Kreuz et al. (1982): $G = 17.06 - 1.32T$, where G is in centimetres and T is in degrees Celsius. In the density-dependent case, the growth was calculated from the multiple regression reported above, which took into account the effect of cohort abundance as well as June temperature. We made the conservative assumption that the deviations from average length of fish in a cohort entering age 4 were dependent only upon the factors affecting age 1 growth; we assumed that subsequent compensatory or depensatory growth was negligible.

The annual growth increments for ages 2–7 were identical for both the density-independent and density-dependent sets of calculations. These increments were the averages of the back-calculated increments at each age from the Astoria population (Kreuz 1978; rounded data reported in Kreuz et al. 1982). Growth increments for ages 8–12 were estimated by first fitting the von Bertalanffy growth equation to the Astoria age 1–7 length-at-age data (Kreuz 1978) and then calculating from this equation the difference in length between successive ages 8–13. The resulting average growth increments are summarized in Table 2.

Results II

Management Implications

The density-independent and density-dependent growth models differ in the forecasted increases in yield and egg production. These increases were for increases in cohort abundance which might be caused by natural events or management actions. At the mean historical temperature of 7.67°C, the density-independent assumption generated a 16.2% larger predicted increase in eggs and a 6.7% larger predicted increase in catch biomass than did the density-dependent scheme (Table 3, Part 1).

In the case where forecasts of yield and egg production were made for a fixed cohort abundance at average temperature, the density-independent model differed only slightly from the density-dependent version (Table 3, Part 2). If we assume that density-dependent growth exists in nature as estimated earlier, than at low age 4 abundance (2406×10^3 fish), the density-independent model underestimated the true yield by 0.9% and egg production by 1.5% (Table 3, Part 2). At high abundance (5724×10^3 fish), density-independent calculations overestimated yield by 3.4% and eggs by 8.1% (Table 3, Part 2).

Discussion

Management Implications

The 1–16% differences between density-independent and density-dependent models in forecasted yield and egg production are probably small in comparison with (1) the changes in those variables caused by interannual variation in catchability or fecundity and (2) errors in field estimates. However, calculations here were done at the average temperature, and in the event that temperature increased simultaneously with increased cohort abundance, the overestimates of yield and eggs by the density-independent version would be much larger. We therefore recommend that density-dependent growth at age 1 be included in models of this stock.

These calculations concerning the implications of density-dependent growth are contingent upon the assumptions that there will be no shift in the natural mortality rate or age-specific vulnerability to the fishing gear as cohort abundance increases and average size of fish decreases. However, if either assumption is violated in nature, the errors in estimates of catch and eggs by density-independent models will likely be even larger than shown here. Age-specific natural mortality will probably increase if fish become smaller, thereby decreasing the number of fish available to be caught and to reproduce.

Density Dependence

Many other fish stocks show density-dependent growth in early life, mainly prior to recruitment stages (Ware 1980). However, in some cases in which growth has previously been shown to be higher when abundance was lower, there was a time trend in both growth and abundance (e.g. Anthony and Fogarty 1985; Iles 1973; other examples in Murphy 1977), leading to an alternative interpretation that some time-trended third variable caused changes in growth and abundance. Such an alternative hypothesis does not apply to our English sole results because neither annual age 1 growth rate nor age 1 biomass is significantly correlated with time (year) ($r = -0.46$, $P > 0.15$ and $r = 0.39$, $P > 0.5$, respectively). We therefore have documented the first known negative feedback mechanism for this population, and in so doing have accounted for significantly more variation in growth rate than the simple temperature relation reported by Kreuz et al. (1982).

It is likely that we have underestimated the probability that there is density-dependent growth of age 1 fish for two reasons.

TABLE 3. Egg production and yield (catch biomass) generated over the life of a cohort of English sole from ages 4–13, under either density-dependent ("Depend.") or density-independent ("Indep.") age 1 growth, and two cohort abundances (numbers of age 4 fish, N_4). All calculations were done at the mean temperature of 7.67°C. Numbers under the "Difference" column are differences between the two quantities to the immediate left. Part 1 compares forecasts of density-independent and density-dependent versions when cohort abundance is *increased*. Here, "% overestimate" is the percentage by which the increase calculated by the density-independent scheme overestimates that calculated by the density-dependent scheme. Part 2 compares the density-independent and density-dependent versions for a *fixed* cohort abundance. Here, "% difference" is the percentage difference between forecasts of density-independent and density-dependent versions, relative to the latter. Letters in parentheses describe how "% overestimate" and "% difference" were calculated.

		Catch biomass		Eggs	
Density type	N_4 (10^3 fish)	Weight (10^6 kg)	Difference (10^6 kg)	Number (10^{13} eggs)	Difference (10^{13} eggs)
		Part 1: increased abundance			
Indep.	2406	0.436	0.602 (*A*)	0.202	0.279 (*C*)
Indep.	5724	1.038		0.481	
Depend.	2406	0.440	0.564 (*B*)	0.205	0.240 (*D*)
Depend.	5724	1.004		0.445	
% overestimate			6.7 $(A-B)/B$		16.2 $(C-D)/D$
		Part 2: fixed abundance			
Indep.	2406	0.436 (*X*)	−0.004	0.202	−0.003
Depend.	2406	0.440 (*Y*)		0.205	
% difference			−0 9 $(X-Y)/Y$		−1.5
Indep.	5724	1.038	3.034	0.481	0.036
Depend.	5724	1.004		0.445	
% difference			3.4		8.1

First, by using age 4 abundance and their mean weight as an index of age 1 biomass, we introduced a source of error in one of the X variates of the multiple regression of age 1 growth; growth and mortality at ages 2–3 undoubtedly vary among years. This measurement error causes our estimated slope of age 1 growth on abundance to be closer to zero than the true slope (Ricker 1973). Second, density-dependent growth of age 1 fish will cause fish from abundant cohorts to be smaller at age 4 than those from less abundant year classes, which would tend to narrow the range of age 4 biomass estimates from the series we used. Regression of the observed growth rates on this narrower range of the X variate would result in a more negative slope.

While we did not have time series data on growth rate at age 0 for our population, other studies have shown variability in growth of English sole during that first year (e.g. Smith and Nitsos 1969). If density dependence affects age 0 as well as age 1 growth, then we may have further underestimated density-dependent age 1 growth by assuming constant age 0 growth in the calculation of the age 1 biomass index. We likely overestimated this biomass index for more abundant cohorts and underestimated it for less abundant groups. In the case of our age 1 regression, this would result in our estimated slope being closer to zero than would actually be the case if true age 1 biomasses were known.

Our regression results were not confounded by using biomass as an independent variate. This is because we used fixed average lengths and weights at age to convert numbers of fish at age into biomass. As indicated in the previous paragraph, this led to conservative tests of the hypothesis of density-dependent growth.

Caution should be taken when using the regression slopes for ages 1–3 shown in Table 1 because, as noted previously, we used the hindcasted biomass of the cohort at age 4 in lieu of data on the true biomass of the cohort at earlier ages. Thus, the magnitudes of the density-dependent regression coefficient for ages 1–3 are not directly comparable with those for ages 4–7, but they are directly comparable with each other.

Our results show that while there is a significant effect of abundance on age 1 growth, the largest effect is due to temperature (temperature partial $r = 0.95$, abundance partial $r = 0.64$). Other studies have also shown a combined effect of physical environment and stock abundance on growth (Iles 1973; Rauck and Zijlstra 1978; Haist and Stocker 1985). In the case of Pacific herring, *Clupea harengus pallasi* (Haist and Stocker 1985), fish abundance and temperature are equally important in changing growth. Omission of the density effect in analyses of that stock's response to management measures would create an even larger bias than we have shown for English sole. It is therefore important to collect the appropriate data and to test for density-dependent processes in the course of doing stock assessments.

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Evaluation of in situ Enclosures for Larval Fish Studies[1]

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Two in situ enclosure designs intended for use in larval fish studies were evaluated for their capability to reproduce and track the physical properties of the surrounding water and to maintain the behavioral characteristics of enclosed organisms. The enclosures, which were constructed of porous material, allowed near instantaneous response to natural variations in temperature, salinity, and dissolved oxygen at all depths. Phytoplankton biomass inside the enclosures was less variable than that observed outside and its size composition was related to the porosity of the material used. Particle sinking rates inside the enclosures were much lower than those previously reported for plastic bags, suggesting a higher degree of turbulence in our enclosures. Newly hatched larval capelin (*Mallotus villosus*) and zooplankton stocked into the enclosures exhibited diel migration of amplitude similar to that observed in the field. Zooplankton were more homogeneously distributed than fish larvae although heterogeneity decreased at night for both taxa. Low cost, ease of handling, environmental reproducibility, and quality of replication provided by the enclosures make them particularly appropriate for replicated experimental studies of the interactions between larval fishes, their predators, and prey.

Deux modèles d'enclos *in situ* destinés à l'étude des larves de poissons ont été évalués quant à leur capacité de recréer et de détecter les propriétés physiques de l'eau ambiante et quant à leur capacité de conserver les caractéristiques du comportement des organismes captifs. Les enclos, construits dans un matériel poreux, permettaient une réponse presque instantanée aux variations naturelles de température, de salinité et de teneur en oxygène dissous à toutes les profondeurs. La biomasse phytoplanctonique à l'intérieur des enclos variait moins que la biomasse ambiante et sa composition par taille était en relation avec la porosité du matériel utilisé. Les vitesses de sédimentation des particules à l'intérieur des enclos étaient nettement inférieures à celles rapportées avec des sacs de plastique, ce qui pourrait indiquer qu'il y a beaucoup plus de turbulence dans nos enclos. Le capelan fraîchement éclos (*Mallotus villosus*) et le zooplancton captifs des enclos manifestaient une migration diel voisine de celle observée sur le terrain. Le zooplancton était distribué de façon beaucoup plus homogène que les larves de poissons même si l'hétérogénéité s'amenuisait la nuit dans les deux taxons. Le faible coût, la facilité de manutention et la possibilité de reproduire les conditions du milieu, avec une bonne précision, rendent ces enclos particulièrement appropriés à la répétition d'études expérimentales de l'interaction entre les larves de poissons, leurs prédateurs et leurs proies.

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In situ estimation of mortality during the early larval stages of fish is made difficult by the sampling effort necessary to cover both spatial and temporal fluctuations in distribution and abundance of natural populations. Even when successful, such approaches yield only averaged results and cannot account for short-term variability resulting from the dynamic nature of the physical environment (Taggart and Leggett 1987). An alternative approach, the tracking of distinct cohorts of larvae while monitoring their abundance over time, may provide more accurate estimates (Shelton and Hutchings 1982; Fortier and Leggett 1985), but the method is not yet well proven. Neither approach yields much in the way of concrete knowledge of the causes of mortality observed. Direct knowledge of the relationships between physical and biological events and larval mortality is best developed through manipulative experiments. Extending the results of small-scale laboratory experiments to field situations has been difficult, however, and larger-scale experiments have been recommended as a possible solution (Solemdal 1981; Oiestad 1982; Houde and Berkeley 1982). The growing use of in situ enclosures to study the early life history of fishes (Table 1) reflects this reality.

The enclosures used to date in various studies on plankton dynamics and pollution have varied in size, volume, wall material, and porosity (Grice and Reeve 1982), but they can be classified into three main types: (1) "nonporous" plastic enclosures which eliminate virtually all water exchange with the surrounding environment, (2) "low-porosity" enclosures which permit limited water exchange with surrounding medium (sailcloth bags), and (3) "high-porosity" enclosures, typically constructed of nylon mesh material, which allow rapid water exchange and selective exchange of plankton.

Any enclosure design will introduce artificialites in the physical and chemical conditions and the biological dynamics of the water column (Banse 1982; Gamble and Davies 1982; Mullin 1982). Nonporous plastic enclosures are particularly prone to

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TABLE 1. Summary of enclosure types and uses for larval fish study.

Enclosure description					
Wall material	*Z*: depth (m) *D*: diameter (m) *V*: volume (m^3)	Duration (d)	Study objectives	Species	Reference
Nonporous bags					
Black polyethylene	*Z*: 2.5 *D*: 0.95 *V*: 1.8	10–32 26 18–42	Growth, survival Growth Survival	Herring Turbot Capelin	Oiestad and Moksness 1981 Rosenberg and Haugen 1982 Oiestad 1985
Transparent PVC bags (Loch Ewe)	*Z*: 19.5 *D*: 4.75 *V*: 310	50–60 60–80 86 56 30	Growth, feeding Growth, feeding Lipids content Otolith growth Growth, feeding	Herring Herring Herring Herring Cod	Gamble et al. 1981 Gamble et al. 1985 Gatten et al. 1983 Geffen 1982 Gamble and Houde 1984
Transparent polyethylene (CEPEX)	*Z*: 23.5 *D*: 10 *V*: 1300	31 45	Growth, feeding Growth	Herring Salmon	Houde and Berkeley 1982 Koeller and Parsons 1977
Porous bags					
White polyester	*Z*: 3.0 *D*: 2.0 *V*: 6.0	76	Survival	Herring	Schnack 1981
White Dacron	*Z*: 5.0 *D*: 1.0 *V*: 3.2	7–10	Growth, survival	Capelin	Leggett 1986
Nitex, 153-μm mesh	*Z*: 5.0 *D*: 1.0 *V*: 3.2	3	Predation mortality	Capelin	Frank and Leggett 1982
Nitex, 505-μm mesh	*Z*: 6.0 *D*: 1.7 *V*: 11.5	14	Survival	Winter flounder	Laurence et al. 1979
Green knotless mesh, 9 and 19 mm	*Z*: 12.0 *D*: 3.1 *V*: 90.0	41	Feeding	Salmon	English 1983
Basins					
Norwegian concrete basin	*Z*: 4.5 *V*: 4400	130–180 73 89 15–20 100	Growth, survival Growth, survival Otolith growth Condition factor Multispecies interaction	Cod Herring Herring Cod Capelin–herring	Ellertsen et al. 1981 Oiestad and Moksness 1981 Geffen 1982 Oiestad 1984 Oiestad 1985
Same as above	*Z*: 5.0 *V*: 2200	11 127	Growth, survival Growth, survival	Turbot Capelin	Rosenberg and Haugen 1982 Moksness 1982

create differences in the physical (temperature, salinity, stratification) and chemical (oxygen levels, pH) properties of the enclosed water relative to the outside conditions by impeding diffusion (Takahashi et al. 1975; Takahashi and Whitney 1977; Steele et al. 1977; Gieskes and Kraay 1982; LeCohu 1982). Most enclosures, regardless of their porosity, significantly reduce horizontal patchiness (Grice et al. 1977, 1980; Oviatt et al. 1977; Stephenson et al. 1984) and restrict the evolution of populations to the vertical dimension. Vertical patchiness varies with enclosure type and plankton species (Takahashi et al. 1975; Grice et al. 1977; Brockmann et al. 1977; Laurence et al. 1979; LeCohu 1982; Harris et al. 1982). The distribution of larval fish in enclosures is poorly described. Gamble and Houde (1984) found that cod larvae were mainly concentrated in the top 7 m of a 20-m-deep nonporous enclosure, but the vertical distribution of fish larvae in relation to diel changes or to different bag size remains unknown.

The characteristics of the "ideal" enclosure should allow (1) good replication among enclosures, (2) good reproducibility of the natural environment, (3) the ability to establish a representative community, and (4) observation of predator–prey relations at natural densities and ratios (Walde and Davies 1984). Replicability and reproducibility have often been inversely related in enclosure work (Kuiper et al. 1983; Hulbert 1984). Researchers who have been interested in studying interactions between trophic levels or in the behavior of larger but scarcer organisms have typically employed large-scale enclosures whose high cost and logistic demands have precluded the use of replicates (Gamble and Davies 1982) and which frequently failed to accurately reproduce the surrounding physical and

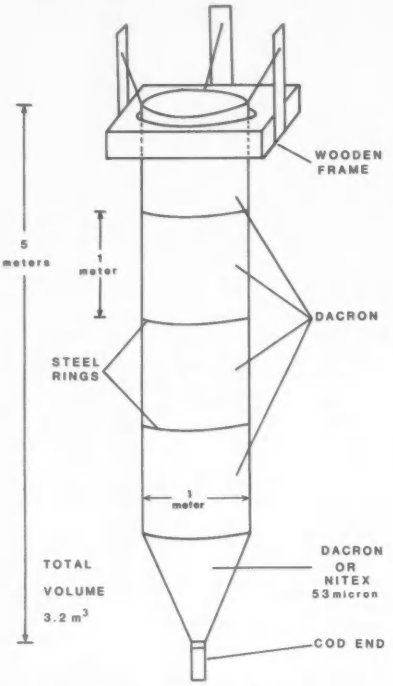

FIG. 1. Specifications of the experimental enclosure.

chemical environment. These workers have justified this approach by arguing that the important consideration is how enclosures duplicate each other and *not* how closely they duplicate the outside environment (Menzel 1977; Menzel and Steele 1978). However, the mounting evidence of the potential effect of short-term physical and biological events on larval growth and survival (Lasker 1975; Frank and Leggett 1982; Fortier and Leggett 1984, 1985) strongly suggests that an ideal enclosure for larval fish studies *should* accurately reproduce the dynamics of the physical and chemical characteristics of seawater and, at the same time, act as a barrier to biological material for manipulation purposes. Careful evaluation of the effect of enclosure design on the characteristics of the physical and biological systems enclosed and on the behavior of enclosed organisms is thus required if results from trophic studies using enclosures are to be applied to natural systems (Solemdal 1981; Hulbert 1984).

In this paper we describe and evaluate an enclosure specifically designed for the study of the growth and mortality of larval fish in situ. We first report the capability of this enclosure to track and reproduce natural variations in the physical properties of the surrounding seawater. We then evaluate the behavioral characteristics of organisms inside the enclosure, relative to their behavior at liberty.

Materials and Methods

The enclosures employed were 1 m in diameter, 5 m deep, and consisted of four cylindrical sections and a conical bottom section, giving a total internal volume of 3.2 m^3 (Fig. 1). The cylindrical sections were constructed from white Dacron sailcloth which has mesh apertures from 15 to 34 μm (average 25 μm) giving a porosity of 0.017. Two types of conical sections were used: one was constructed of Dacron and the second of 53-μm Nitex having a porosity of 0.265. A 10-cm band of raw (untreated) canvas was sewn to the external side of the enclosures at 1-m intervals. A 6-mm solid steel ring was lashed to each canvas band to support the enclosure walls. The enclosures were supported at sea in pairs by a floating wooden frame anchored to the sea bottom. The frames were joined in pairs, thereby creating a four-enclosure platform on which two persons could stand and work with ease. A 10-cm-diameter PVC cod-end collector with a side window of desired mesh size was attached to the bottom of the enclosure to facilitate recovery of the experimental organisms at the end of the experiment. To prevent the organisms from becoming trapped in the cod-end during the experiment, a tight-fitting plug was installed over the cod-end from the inside of the enclosure before deployment. Prior to lifting the enclosure at the end of an experiment, the plug was pulled using a line running to the surface. We allowed the enclosures to fill by diffusion of water through the walls. This required about 1.5 h for the full Dacron type and less than 5 min for the Nitex cone type.

The Dacron/Nitex enclosures were hauled by hand with two persons holding one section at a time and a third rinsing the inside walls. Rinsing seawater was continuously pumped from the surrounding waters and passed through a series of three filters of 153, 53, and 20 μm. The hauling procedure was completed in 15 min. The Dacron enclosures were lifted with block and tackle fixed to a portable wooden A-frame installed on the floating platform. Once one section was out of the water and rinsed, the block and tackle was attached to the next steel ring and the rinsed section was folded down. This process was repeated until the conical section holding the 20-μm cod-end was out of the water. The hauling operation required about 1.5 h per enclosure. The cost for one complete enclosure of either design was approximately Can$1500.

Diffusibility Experiment

Two enclosures of each design were moored in the nearshore waters at Bryant's Cove, Newfoundland (47°40′36″N, 53°11′24″W) on June 20, 1983, at 15:00 local time (11:00 GMT). Sampling was initiated at 18:00 EST and was repeated every 3 h for 24 h and then on a daily basis (at approximately 9:00 local time) for 6 consecutive days. Temperature, salinity, and dissolved oxygen were measured at depths of 0, 1, 2, 3, 4, and 5 m both inside and outside the enclosures. A Parr diaphragm pump (flowrate of 3 L/min) was used to collect 500-mL water samples at depths of 0.5, 2.5, and 4.5 m inside and outside the enclosures. These samples were used to construct vertical profiles of fluorescence, chlorophyll *a*, and particulate matter. Relative fluorescence was measured with a Turner Design model 10 fluorometer and chlorophyll *a* was determined by fluorometric techniques (Parsons et al. 1984). Replicate (250 mL) subsamples were preserved with 2% unbuffered formaldehyde seawater solution for subsequent particulate matter analysis. Particle concentrations in 20 size classes ranging from 2.0 to 161 μm equivalent spherical diameter (e.s.d.) were determined with a Coulter model TA-II counter fitted with 100- and 400-μm-aperture tubes. Prior to counting, the preserved sample was gently but thoroughly mixed, filtered through a 153-μm filter, and split into two equal fractions. One fraction was processed on the 400-μm tube at a flowrate of 0.56 mL/s until 2.0 mL of the sample had

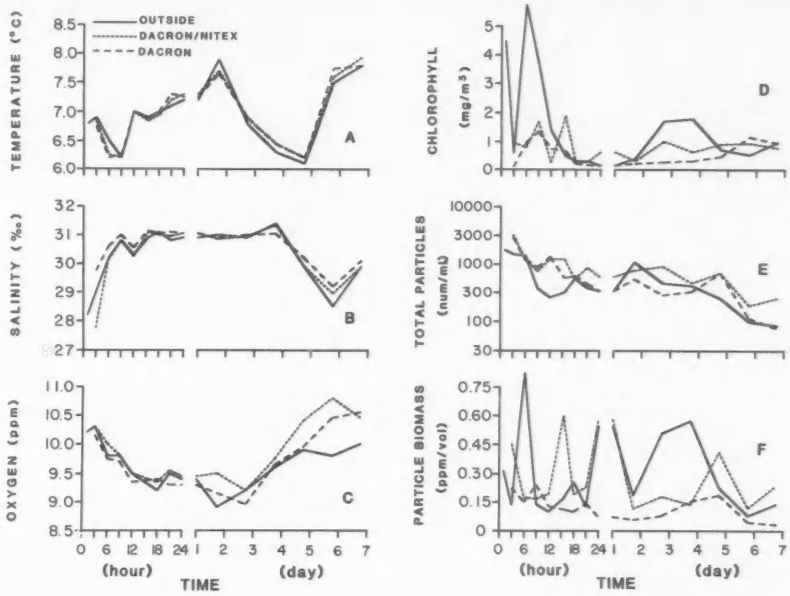

FIG. 2. Temporal variation in physical and biological properties at middepth (2 m) inside and outside the experimental enclosures.

been counted. The second fraction was sieved again on a 43-μm filter and counted with the 100-μm-aperture tube using the manometer (0.5 mL) mode of operation (Sheldon and Parsons 1967a). Replicate counts were made on each sample and the resulting counts were averaged. Total particle biomass expressed in parts per million per volume was estimated from the sum of volumes of each size class.

Behavior Experiment

The diel vertical distribution of yolk-sac capelin (*Mallotus villosus*) larvae was examined in three experiments conducted in 1982, 1983, and 1984. Four full Dacron enclosures were used in 1982. Four Dacron/Nitex enclosures were employed in 1983 and one in 1984.

Larvae were collected from the capelin spawning beach at Bryant's Cove and stocked into each enclosure (1500/enclosure in 1982 and 1984; 2500 in 1983). A separate sample of not less than 100 larvae was collected at the same time and these larvae were measured to the nearest 0.1 mm total length (TL) to determine the initial size of the stocked larvae. In 1984, wild zooplankton collected from the surrounding seawater with a large-volume plankton pump (Taggart and Leggett 1984) and filtered on 1-mm-screen filter was added to the enclosures with the larvae.

Vertical distribution of larvae and plankton was assessed by pump sampling from within predetermined layers: surface (0−1 m), middepth (2−3 m), and bottom (4−5 m). All samples (74 L) were collected with a Jabsco submersible pump having a mean flowrate of 37 L/min and were preserved immediately in a 4% formaldehyde seawater solution buffered with sodium borate. Zooplankton counts were made with a Coulter model TA-II particle counter using a 2000-μm-aperture tube. This yielded counts for plankters of 10 size classes ranging from 80 to 520 μm e.s.d.

Data on the vertical distribution of both zooplankton and fish larvae were expressed as the proportion taken at depth for each sampling session. Arc-sine transformation (Sokal and Rohlf 1981) was applied on proportion data prior to an analysis of variance of differences between years and enclosures. We tested for deviations from a hypothetical distribution having equal proportions at each depth with a chi-square test (Sokal and Rohlf 1981).

Results

The physical and biological properties of the waters in Bryant's Cove are temporally dynamic on a short time (2−6 d) scale (Taggart and Leggett 1987). During offshore winds, deep, cold, high-saline water upwells in the cove. This water mass is characterized by relatively low abundance of particles in the <150 μm size classes. During onshore winds, warm surface waters are advected onshore. These waters are characterized by high concentrations of particles <150 μm (Frank and Leggett 1982). On average, the chlorophyll and particle concentrations in the Cove under either wind condition were much lower than those reported for estuarine (Chanut and Poulet 1982) or other coastal waters (Sheldon and Parsons 1967b; Maysaud et al. 1984) but more similar to oligotrophic oceanic waters (Flos 1984; Sheldon 1984). Evidence of these rapid temporal changes in the physical and biological characteristics of the waters outside the enclosures is given in Fig. 2.

Temperature, Salinity, and Oxygen

The waters contained in both enclosure designs responded almost immediately to outside fluctuations in temperature, salinity, and dissolved oxygen (Fig. 2A, 2B, 2C). Correlation analysis for the hourly portion of the record (hour 1−24) revealed highly significant ($p < 0.001$) coefficients between

TABLE 2. Percentages of the sum of squares for main factors and interactions from the three way ANOVA. Chlorophyll and particle data were normalized by logarithmic transformation (log $X + 1$) prior to the analysis. Temp. = temperature; Sal. = salinity; DO = dissolved oxygen; RF = relative fluorescence; Chl = chlorophyll *a*. Significant variances (*F*-test): underlined = $p < 0.0001$; $^{**}p < 0.01$; $^{*}p < 0.05$.

Factor	Temp.	Sal.	DO	RF	Chl	Particle number	Particle biomass
Time	70.34	78.24	56.92	38.34	24.77	47.02	7.90**
Enclosure	0.44	3.83	1.72	15.90	5.39	3.76	6.95
Time × enclosure	0.50	8.06	4.17	11.28**	12.39*	6.05	10.67**
Depth	20.66	4.11	23.23	0.30	0.17	2.69	2.59**
Time × depth	6.10	2.09	3.98	7.17	16.64**	7.58	5.49
Enclosure × depth	0.34	0.58	1.88	0.20	1.76	2.00**	1.66
Time × enclosure × depth						5.98	8.07

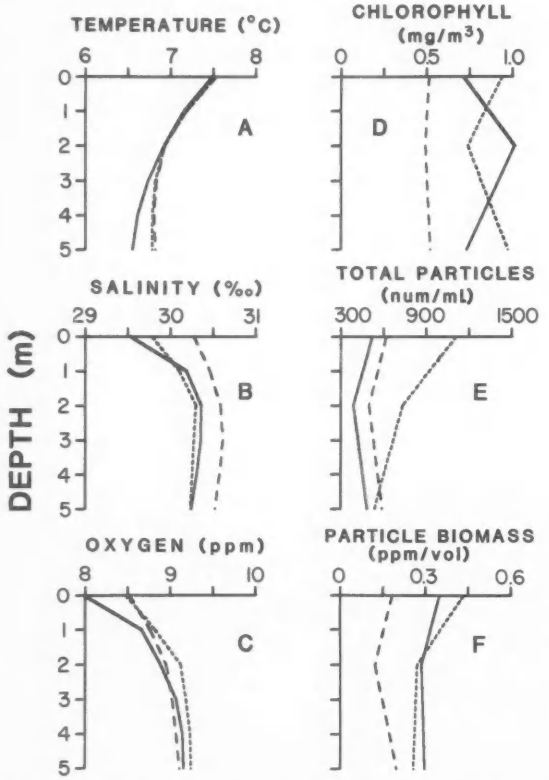

FIG. 3. Vertical profiles of physical and biological properties averaged over time inside and outside the enclosures. Curves are as defined in Fig. 2.

outside and inside conditions. When lags of 3 and 6 h were introduced, coefficients became nonsignificant ($p > 0.1$), indicating a time response of less than 3 h. The effects of time, enclosure, and sample depth on the overall variability of each parameter were tested by a three-way analysis of variance

TABLE 3. Results of the GT2 multiple comparison test between enclosure types and outside. O = outside, D = Dacron; N = Dacron with 53-μm Nitex. Underlined types are not significantly different ($p > 0.05$). Enclosure types are ordered from low to high values (from left to right) for each variable.

Variable	Sampling depth (m) 0			2			5		
Temperature	O	D	N	O	D	N	O	N	D
Salinity	O	N	D	N	O	D	N	O	D
Oxygen	O	N	D	O	D	N	D	O	N
Relative fluorescence	D	N	O	D	N	O	D	N	O
Chlorophyll	D	O	N	D	N	O	D	O	N
Particle number	O	D	N	O	D	N	O	N	D
Particle biomass	D	O	N	D	N	O	D	N	O

(Table 2). The time effect explained 57−78% of the overall variance; less than 5% was explained by enclosure effects.

The internal and external vertical profiles of each physical variable, based on time averaged data, were similar (Fig. 3A, 3B, 3C). The differences between enclosures at depths of 0, 2, and 5 m were tested by an analysis of variance followed by a GT2 multiple comparison test (Sokal and Rohlf 1981) (Table 3). In order to filter the temporal component of variability in the data, the analysis was performed on the residual values between the observed values of an enclosure and the mean value of all enclosures for each sampling time. On average, temperature differences were observed mainly at the bottom. The waters enclosed by the full Dacron enclosures were consistently, but only slightly, more saline (0.3‰) than the surrounding waters at all depths, and dissolved oxygen levels were slightly higher (0.15−0.5 ppm) inside the Dacron/Nitex type. A Spearman rank correlation used to test whether the temporal events at 2 m shown in Fig. 2 were also observed at other depths inside the enclosures reveals very strong correlation ($p < 0.001$) between depths for all physical variables.

Chlorophyll and Particulate Matter

Chlorophyll *a* concentrations, particle number, and biomass (Fig. 2D, 2E, 2F) were all more variable outside than inside the

TABLE 4. Sinking rate estimates for total particles lesser and greater than 10 μm esd in both replicates of each enclosure type. Decline rate (K_s) and sinking rate (S) were calculated for each depth. Regression coefficients (r^2) and probability level of significance (p) of equation 2 to calculate K_s are also tabulated.

		Less than 10 μm				Greater than 10 μm			
Enclosure type	Depth (m)	r^2	p	K_s (h^{-1})	S (m/d)	r^2	p	K_s (h^{-1})	S (m/d)
Dacron	0	0.70	0.0002	−0.0129	0.266	0.15	0.174	−0.0037	0.086
	2	0.63	0.0006	−0.0166	0.329	0.25	0.072	−0.0045	0.102
	5	0.15	0.17	−0.0067	0.148	0.17	0.133	+0.004	—
	0	0.68	0.0003	−0.0173	0.340	0.22	0.09	−0.0056	0.126
	2	0.44	0.0095	−0.0119	0.248	0.08	0.32	−0.0019	0.045
	5	0.48	0.0056	−0.0124	0.257	0.11	0.23	−0.0037	0.085
Dacron/ Nitex	0	0.60	0.0011	−0.0142	0.289	0.20	0.107	−0.005	0.113
	2	0.34	0.027	−0.0095	0.204	0.02	0.667	−0.0015	0.036
	5	0.19	0.114	−0.0044	0.010	0.04	0.524	−0.0033	0.075
	0	0.32	0.035	−0.0097	0.208	0.09	0.30	−0.0034	0.079
	2	0.75	0.0001	−0.0111	0.234	0.31	0.037	−0.0076	0.167
	5	0.42	0.013	−0.0123	0.256	0.05	0.44	−0.0022	0.053

enclosures. The variance explained by the time effect was lower (8−47%) than was the case for the physical variates, and the enclosure effect (either alone or its interactions) was more pronounced (Table 2). There were no significant ($p > 0.1$) correlations between values of the biological variables measured outside and inside the enclosures. Time lags of up to 9 h did not significantly improve the coefficients ($p > 0.1$). This suggests that the biological characteristics of the enclosures were not directly related to earlier external events. Particle and chlorophyll *a* concentrations were generally homogeneous with depth, but were lower inside the full Dacron enclosures than in the Dacron/Nitex types (Fig. 3D, 3E, 3F; Table 3) as was expected given their lower porosity. Spearman rank correlations indicated significant ($p < 0.001$) temporal correspondence between depths for chlorophyll *a* and particle concentrations but not for particle biomass ($p > 0.1$).

Total particle number declined sharply with time, but particle biomass remained relatively constant (Fig. 2E, 2F). This suggests that the size spectrum of particles varied over time. The size distribution of particles was dominated by a maximum in abundance at 40−100 μm (Fig. 4) which probably corresponds to large diatoms (Harris et al. 1982; Takahashi et al. 1982). One possibility is that the general decline in particle numbers with time observed in the enclosures was due to sinking. The sinking rate necessary to produce this decline was estimated independently for particles smaller and larger than 10 μm. These rates were derived for each stratum sampled using equation 1 (Reynolds and Wiseman 1982):

$$(1)\quad S = Z_m \cdot (1 - (1/e^{-K_s}))$$

where S is the sinking rate (metres per day), Z_m is the sampling depth interval (1 m), and K_s is the decline rate in numbers calculated as follows:

$$(2)\quad K_s = \frac{\log_e(N_{t-1}/N_t)}{t}$$

where N_{t-1} is the number of particles at time $t + 1$, N_t is the number of particles at time t, and t is the time interval (hours) between samples. The average sinking rates of particles

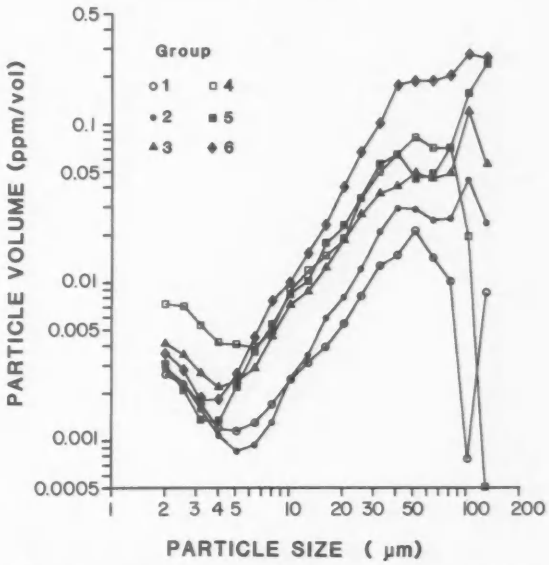

FIG. 4. Averaged particle size distributions of the six size spectra identified by cluster analysis.

<10 μm from the upper (0 and 2 m) layers of the full Dacron and the Dacron/Nitex enclosures were 0.297 and 0.235 m/d, respectively (Table 4). Average sinking rates of particles >10 μm in the Dacron and the Dacron/Nitex enclosures were 0.089 and 0.098 m/d, respectively. These estimates did not differ significantly from 0 (Table 4).

To evaluate the effect of the porosity of the enclosure walls on the plankton size structure within the enclosures, the 210 particle size spectra obtained from the Coulter data were reduced to six similar size spectra by cluster analysis (Fig. 4). Evaluation of the significance of these six spectra, and their distribution in the two enclosures types and in situ (Table 5),

TABLE 5. Partitioning (%) of particle size spectra as defined by cluster analysis and discriminant analysis (see Fig. 4).

Enclosure type	Depth (m)	Particle size spectra group					
		1	2	3	4	5	6
Dacron	0	17.8	11.9	1.2	2.4	0	0
(n = 84)	2	21.4	9.5	1.2	1.2	0	0
	5	10.7	19.0	2.4	1.2	0	0
	All	50.0	40.5	4.8	4.8	0	0
Dacron/	0	11.9	10.7	3.6	0	0	2.4
Nitex	2	15.5	8.3	7.1	0	1.2	1.2
(n = 84)	5	7.1	19.0	3.6	0	0	1.2
	All	34.5	38.1	14.3	0	1.2	4.8
Outside	0	9.5	7.1	7.1	4.8	2.4	2.4
(n = 42)	2	16.7	7.1	2.4	2.4	4.8	0
	5	9.5	19.0	0	0	2.4	2.4
	All	35.7	33.3	9.5	7.1	9.5	4.8

was achieved by discriminant analysis as suggested by Green (1979). The six size spectra differed primarily in the abundance of particles >5 μm (Fig. 4). Full Dacron enclosures, which had the lowest porosity, were dominanted by groups 1 and 2 which had lower abundances of particles at all size classes. Size spectra 3–6, which were characterized by a greater abundance of particles at all size classes, were virtually absent inside the Dacron type although they were observed both outside and inside the Dacron/Nitex enclosures. It should be emphasized that the size classes provided by the Coulter counter are equivalent spherical diameters, which are derived from volume estimates. By this measure, long slender particles, spherical diameter particles, or even small spheres with long spines can have equal e.s.d. values. This explains the observed presence of particles larger than 53 μm e.s.d. in the Dacron/Nitex enclosures. Large particles (groups 3–6) were not restricted to the Nitex bottom region where they entered, but were quickly entrained within the full water column. No group separation due to sampling depth was evident in the discriminant analysis. Moreover, specific particle size groups did not persist over time in the Dacron/Nitex enclosures. Rather, they were repeatedly flushed and replaced by groups characteristic of the water mass surrounding the enclosures.

Larval Distribution

Yolk-sac capelin larvae exhibited diel vertical migration within the enclosures. The number of larvae caught relative to the number present in the enclosure at the time of sampling averaged 5.7% (SE 0.3%) and was independent of sampling time. Due to the different number of larvae initially stocked in the enclosures and due to removal of larvae by sampling, the number of larvae sampled at each time varied between 32 and 224 (average 88 and SE 4.6). Larvae were proportionally more abundant near the surface and near the bottom of the enclosures (Fig. 5); few larvae were sampled at middepths. Despite the differences in temperature, mean daily solar radiation, and numbers initially stocked (Table 6), the proportion of larvae found at depth did not differ between years (Table 7, YEAR effect, $p > 0.1$). The pattern of larval distribution in the full Dacron enclosures (1982) and Dacron/Nitex enclosures (1983, 1984) was thus similar. The pattern of diel migration did, however, vary between years (Table 7, interaction of YEAR and TIME, $p < 0.001$) and appears to have been related to the corresponding presence or absence of zooplankton in the enclosures. In 1982 and 1983, when no zooplankton was stocked, the proportion of larvae sampled near the surface exhibited two daily maxima centered on dawn and dusk and two minima near midnight and midday (Fig. 5). The proportion of larvae at the 5-m sampling depth showed a reciprocal pattern being greatest a midnight and noon. In 1984, when both zooplankton and larvae were stocked, larvae aggregated near the surface during daylight hours with no sign of midday minimum. The proportion of larvae in the 5-m zone was greatest at night.

The effect of bag dimension on the vertical distribution of larvae was examined in 1984 by launching a modified enclosure with a depth of 9 m and a volume of 6.4 m^3. Wild zooplankton was added to the enclosures with the larvae. Larvae were proportionally more abundant at surface during daylight hours and the proportion near the bottom was maximal at night (Fig. 5). The pattern of diel migration in the 9-m enclosure was thus similar to that observed in the 5-m enclosure also deployed in 1984.

Larvae were more homogeneously distributed during darkness relative to the daylight period. Except for two occasions (0 h in 1983 and 2 h in 1984 – 9 m), the vertical distribution of capelin larvae in the enclosures differed significantly from a uniform distribution ($\chi^2 > 5.99$, $p < 0.05$ for 5-m enclosures and $\chi^2 > 9.45$, $p < 0.05$ for 9 m). The probability level of the χ^2 values was, however, higher ($p < 0.001$) during the day as opposed to the night ($0.001 < p\ 0.05$) (Fig. 5). Heterogeneity (variance/mean ratio) was significantly greater in the 9-m enclosure relative to the 5-m enclosure during daytime only (F = 41.4, $p < 0.0001$).

Zooplankton Distribution

The vertical distribution of three size groups (group 1: 80–150 μm, group 2: 150–300 μm, and group 3: >300 μm) of zooplankton stocked into the enclosures in 1984 revealed diel movement which resulted in proportionally higher densities near the surface at night (Fig. 6). The greatest migration amplitude was exhibited by the largest (>300 μm) size groups. This group also showed a night surface peak that was both narrower and earlier than that of the two other groups. Day–night changes in proportion were significant at the surface and at the bottom for both the 5- and 9-m enclosures (Table 8). Chi-square analysis indicated that the zooplankton was more uniformly distributed at night than during daytime in both enclosures sizes. This effect was, however, more pronounced for small and medium sized organisms. Heterogeneity (variance/mean ratio) was independent of bag dimension for all size groups (F-test, $p > 0.50$).

Discussion

Our data indicate that the physical and chemical conditions inside both enclosure designs closely mirror those in situ. The time response to changes in in situ conditions was almost instantaneous. This contrasts sharply with the performance of plastic bags which act as "lowpass" filters of in situ temperature having a lagged time response of between 12 and 24 h (McAllister et al. 1961; Steele et al. 1977; Sonntag and Parsons 1979). Both enclosure types also had vertical temperature and salinity profiles similar to those occurring outside. This differs dramatically from the pronounced and stable stratified conditions which typically occur in plastic bags (Takahashi and

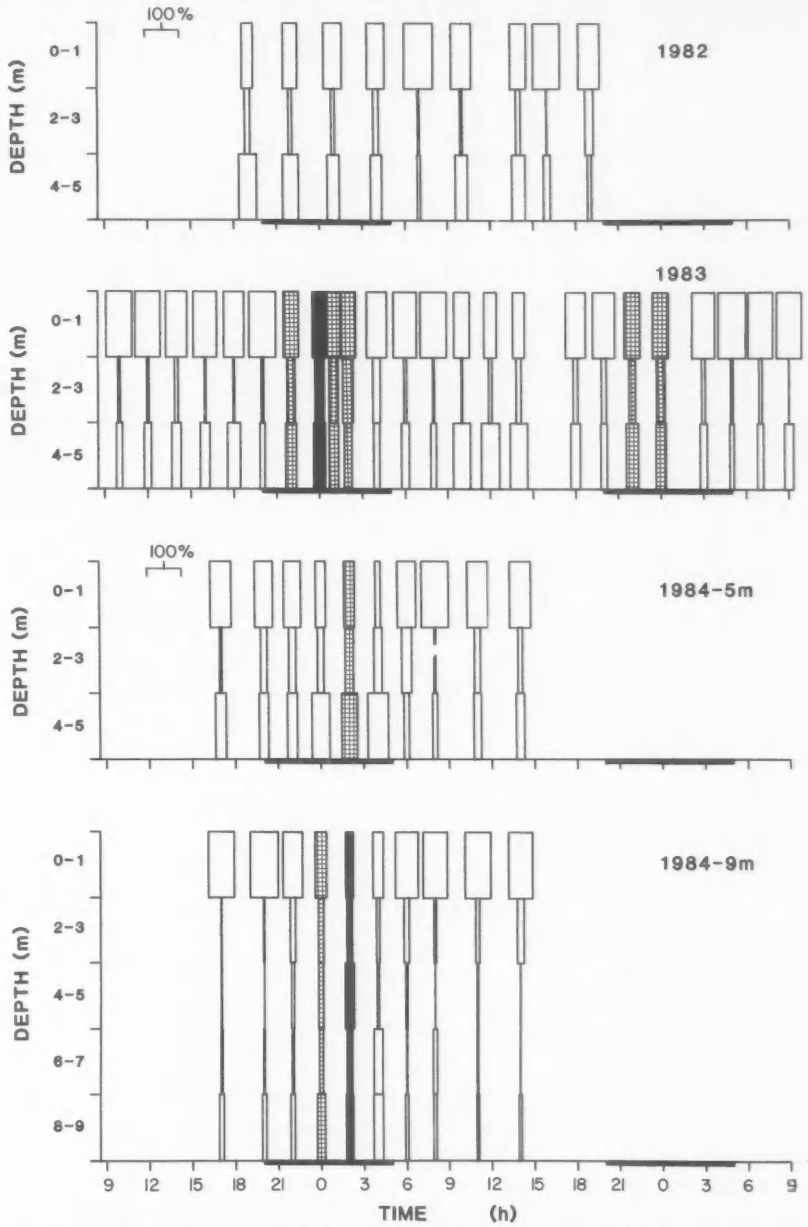

FIG. 5. Vertical distribution of capelin larvae in the enclosures. Histogram width represents the percentage of larvae collected in a given stratum during each sampling session. Deviations from a uniform distribution (chi-square test) are presented. Open: $p < 0.0001$; hatched: $0.0001 < p < 0.05$; solid: $p > 0.05$.

Whitney 1977; Sonntag and Parsons 1979). The similarity in O_2 levels inside and outside the enclosures also differs markedly from the supersaturation (McAllister et al. 1961; Takahashi and Whitney 1977) or undersaturation (Gieskes and Kraay 1982) conditions typical of nonporous bags.

Reduced vertical mixing is considered to be the most important problem of plastic bag enclosures. Boyce (1974) cautioned that the similarity of vertical temperature profiles inside such enclosures with in situ profiles can result from lateral heat transfer and cannot be interpreted as evidence of vertical mixing. Steele et al. (1977) showed that vertical eddy diffusivity decreases with increasing bag size. They suggested that vertical

TABLE 6. Summary of conditions during vertical distribution experiments.

	Experiment			
	1982	1983	1984 − 5 m	1984 − 9 m
Enclosure type	Dacron	Dacron/ Nitex	Dacron/ Nitex	Dacron/ Nitex
Dimension (m)	1 × 5	1 × 5	1 × 5	1 × 9
Volume (m^3)	3.2	3.2	3.2	6.4
Replicates	4	4	1	1
Initial number of larvae	1500	2500	1500	1500
Duration (h)	22	48	21	21
Larvae removed by sampling (%)	43.7	56.1	34.9	32.1
Initial mean length (mm) of larvae (SD)	4.71 (0.30)	4.85 (0.31)	4.77 (0.31)	4.77 (0.25)
Mean water temperature (°C) (SD)	5.5 (.57)	11.0 (.59)	11.4 (1.27)	11.4 (1.27)
Total daily radiation (MJ/m^2)	15.98	20.45	23.71	23.71

TABLE 7. Analysis of variance of proportion of larvae at each depth inside the enclosures.

		Sampling depth (m)					
		0		2		5	
Source of variation	df	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Year	2	1.71	0.187	2.18	0.119	1.47	0.241
Time (day/night)	1	17.86	0.0001	40.53	0.0001	3.25	0.092
Enclosure (within year)	6	1.13	0.352	1.81	0.108	1.60	0.153
Year × time	2	7.11	0.001	6.13	0.001	9.39	0.0002
Time × enclosure	5	0.25	0.941	1.54	0.185	0.21	0.957

mixing in plastic bag enclosures is probably due to wall movement and thermal forcing, both of which are inversely related to bag dimension. von Brockel (1982) hypothesized that enclosures constructed of porous material would alter the energy profile of enclosed systems much less than is the case for impermeable enclosures. Our data support this hypothesis. In our enclosures, both chlorophyll and particle profiles were virtually homogeneous vertically in both the full Dacron and the Dacron/Nitex types. Moreover, the profiles were similar to those observed in situ (Fig. 3; Table 4). In plastic enclosures, the sinking rate of particles, which has been often used as an index of turbulent mixing, is rapid during the first 2 wk after filling and is positively related to particle size and bag dimension (von Brockel 1982; Bienfang 1982). The sinking rates calculated for both of our enclosure types (0.089−0.297 m/d) were much lower than those previously reported for plastic bags (0.32−5.0 m/d: von Brockel 1982; Bienfang 1982). The above lines of evidence suggest that the wall permeability and the smaller size of our enclosures enhanced internal turbulence, thereby creating conditions more typical of those found in situ.

Chlorophyll levels inside our enclosures remained low over the 1-wk duration of our experiments and never reached the peaks observed in situ (Fig. 2D). Previous work with plastic bags has also shown chlorophyll to remain relatively close to the initial concentration for approximately 1 wk after filling, although significant increases commonly occur after that time (McAllister et al. 1961; Takahashi et al. 1975; Brockmann et al. 1977; Kuiper 1977; Kuiper et al. 1983). The differences in chlorophyll and particle concentrations we observed between the Dacron and the Dacron/Nitex enclosures are consistent with the porosity of the material used. The full Dacron enclosures appear to create an effective biological barrier, while the Dacron/Nitex design allows limited exchange of small biological material with the outside environment.

In nature, newly hatched capelin larvae are typically restricted to the upper (0−20 m) layer of the ocean. Yolk-sac capelin larvae migrate on a diel basis by moving toward the surface at twilight and being most abundant there at night (Fridgeirsson 1984; Taggart and Leggett 1987). The diel migration exhibited by capelin larvae in the enclosures indicates that the migratory behavior they typically exhibit in situ was not disturbed. However, the pattern of vertical migration we observed in our enclosures was inverse to that reported in situ. In the enclosures, the larvae were distributed more homoge-

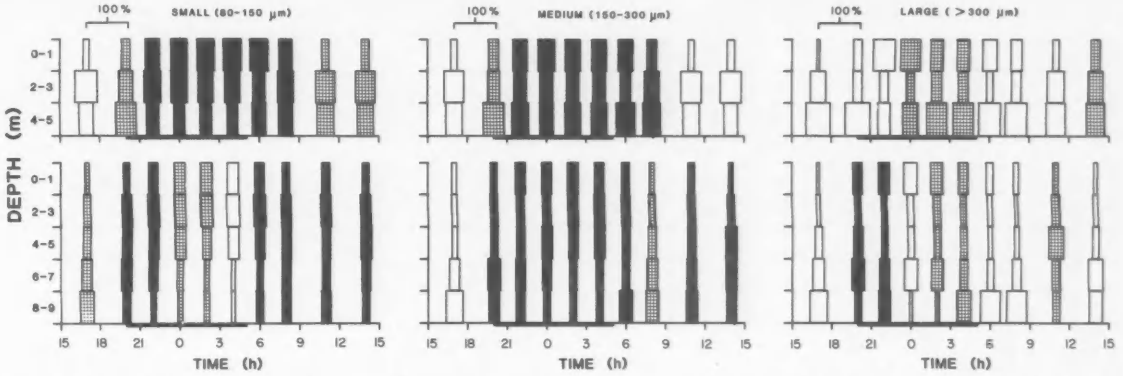

FIG. 6. Vertical distribution of three size groups of zooplankton inside 5- and 9-m-long Dacron/Nitex enclosures. Histogram width represents the percentage of zooplankton collected in a given stratum during each sampling session. Deviations from a uniform distribution (chi-square test) are presented. Open: $p < 0.0001$); hatched: $0.0001 < p < 0.05$; solid: $p > 0.05$.

TABLE 8. Analysis of variance of the three size groups of zooplankton inside the enclosures in 1984: F-values for the effect of time (day/night) for each sampling depth. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Group size	Enclosure size (m)	Sampling depth (m)				
		0	2	5	7	9
Small	5	7.12*	0.49	13.60**		
(80–150 µm)	9	12.91**	5.54*	2.49	5.84*	13.48**
Medium	5	11.87**	2.30	2.53		
(150–300 µm)	9	36.57***	6.31*	2.53	5.08	16.46**
Large	5	7.07*	1.71	4.98		
(>300 µm)	9	15.94**	1.55	0.41	0.05	2.54

neously at night and were proportionally more concentrated in the surface layer during the day (Fig. 5). Gamble and Houde (1984) also found that recently hatched cod larvae were mainly concentrated in the top layer of an enclosure, while in nature they are typically found in deeper strata (10–30 m) (Fridgeirsson 1984; Solemdal and Ellertsen 1984; Ellertsen et al. 1984; Lough 1984). Although the vertical movements exhibited by capelin larvae in enclosures are consistent with laboratory results for herring and red sea bream larvae whose activity was random in total darkness but vertically surface oriented in daytime (Woodhead and Woodhead 1955; Tsuda and Sakamoto 1983), there is no obvious explanation for the observed difference between enclosure and in situ larval distribution. The diel vertical distribution of larvae (Fig. 5) was unrelated to the diel distribution of the small (80–150 µm) zooplankton (Fig. 6) which represent potential food items for newly hatched larvae (Courtois and Dodson 1986). Fortier and Leggett (1984) have also shown the vertical distribution of capelin larvae and their principal prey to be unrelated in situ. At night the distribution of larvae was inversely related to that of medium (150–300 µm) and large (>300 µm) sized zooplankters.

The vertical migration of zooplankton to the surface at night observed in our enclosures is consistent with previous observations by Kuiper et al. (1982) in a 3-m-deep enclosure. The greater migration amplitude of larger zooplankton has also been reported for zooplankton in CEPEX bags by Harris et al. (1982). Our results indicated that patchiness increases with animal size as previously reported by Grice et al. (1977). However, we also found it to vary on a diel basis, becoming most pronounced during the day.

The migratory behavior of both fish larvae and zooplankton was unchanged in the 9-m enclosure. Zooplankton patchiness did not differ between the 5- and 9-m enclosures, but the distribution of fish larvae was significantly more heterogeneous in the 9-m enclosure during daylight periods. This reinforces the evidence that larval distribution was not directly associated with their potential food items.

The fact that the distribution of capelin larvae was, on average, more patchy than that of zooplankton and that the larvae did not directly follow their potential food items (small zooplankton) is puzzling given the several hypotheses on the importance of feeding and food patches for larval survival (Lasker 1975; Theilacker and Dorsey 1980; Fortier and Leggett 1984; Houde and Lovdal 1985). Much needed tests of these hypotheses could easily be conducted by manipulating the size spectrum of zooplankton available to larvae. We suggest that enclosures may be useful tools for such experiments.

We conclude that the two enclosure types evaluated in this study have significant potential for studies of the early life history dynamics of marine fish. In addition to the accurate reproducibility of the physico-chemical conditions that larvae normally encountered at sea, the enclosures acted as effective biological barriers as required for manipulation purposes. Replicability of both the physical and biological properties and the natural behavior of enclosed organisms between enclosures and between years was also excellent. In comparison with laboratory experiments, enclosures confer the advantages of

larger scale where the natural ratios of predator and prey can be achieved.

The choice of an enclosure design is primarily determined by the aims of the experiment, and no "ideal" enclosure exists per se. The choice of design is analogous to the choice of a sampling gear which depends on the type of organisms to be studied (Green 1979). Recent research on larval fish contained in enclosures (Table 1) has been realized primarily in large enclosures and in concrete basins principally because the objectives were long term. As we have previously noted, these designs virtually preclude replication of experimental treatments and make difficult the design of short-term experiments.

The relatively low cost, ease of handling, and reproducibility provided by our enclosures make them particularly appropriate for short-term replicated experimental studies of the interactions between larval fishes, their predators, and prey. Enclosures of the design we describe have also been successfully used for short-term experiments on larval mortality induced by macrozooplankton predators (Frank and Leggett 1982; Y. de Lafontaine and W. C. Leggett, in prep.). The extent to which the properties of our enclosures will persist over longer periods remains unknown. However, Kuiper et al. (1983) found no difference in the temporal development of phytoplankton in plastic bags of different sizes (1.5–30 m^3) over a 4-wk period, and studies on fish larval growth and survival in relation to feeding regimes (Schnack 1981; Leggett 1986) have been successfully conducted in enclosures similar to the ones described in the present study. This suggests that these enclosures also have potential for longer-term experiments.

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Development and Performance of an Alaska Steeppass Fishway for Sockeye Salmon (*Oncorhynchus nerka*)

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A major introduced run of sockeye salmon (*Oncorhynchus nerka*) at Frazer Lake, Kodiak Island, Alaska, is perpetuated by an Alaska steeppass fishway at a 10-m-high falls on the outlet river. Experimental modifications of the fishway, which was inadequate by 1970 as the salmon run increased, improved salmon entry and passage. Sockeye passage over 4 yr was nearly equivalent in a steeppass of 60 m continuous length (22% slope) and an adjoining steeppass with three resting pools, but significant year-to-year differences in passage occurred between steeppasses. Resting tanks were beneficial for holding slower or descending salmon without blocking passage of other salmon. An average entry of 142 sockeye in 15 min was achieved by guiding all salmon along a weir to an entrance tank with a vertical slot orifice. Chutes installed in the entry end of the steeppasses created a high-velocity stream for attraction and decreased salmon dropout from the entrance tank. Salmon passage in the original steeppasses with three resting pools was greater than in new (1979) steeppasses with a single resting pool. In 1985, sockeye passage of 2925/h and 46 806/d exceeded fishway capacity requirements.

C'est grâce à une échelle à poissons à pente raide de type Alaska installée à l'émissaire du lac Frazer (île Kodiak, Alaska), où les chutes atteignent 10 m de hauteur, que l'importante remonte de saumon rouge (*Oncorhynchus nerka*) ensemencé se perpétue. Des modifications expérimentales de l'échelle à poissons qui, dès 1970, s'est révélée inadéquate vu l'accroissement du nombre de géniteurs amontants, ont facilité l'entrée et le passage des saumons. Le nombre de poissons qui ont utilisé une passe continue de 60 m de longueur (pente de 22 %) et une passe avoisinante avec trois bassins de repos était presque le même pendant 4 ans, mais des différences significatives du nombre ont été notées entre les passes d'une année à l'autre. Les bassins de repos se sont révélés avantageux pour les saumons dévalants ou lents; de plus, ceux-ci n'entravaient pas le déplacement des autres. En guidant à l'aide d'un filet tous les saumons vers un bassin d'entrée à ouverture en créneaux verticale, on a obtenu un passage moyen de 142 poissons par 15 min. Un fort courant a été créé à l'entrée de l'échelle grâce à l'installation de plans inclinés; ce courant a attiré les saumons et réduit le nombre de poissons qui ont quitté le bassin d'entrée. Le nombre de saumons qui ont utilisé les premières échelles à trois bassins de repos était plus élevé que dans le cas des plus récentes échelles (1979) à bassin unique. En 1985, 2 925 saumons rouges/h (46 806/j) ont remonté l'échelle, une valeur supérieure aux exigences nominales de l'installation.

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Barrier falls often block the natural ascent of anadromous salmon into rivers and lakes. Construction of fishways for passage of salmon over the falls, combined with salmon introduction, provides a method of establishing new salmon runs and utilizing otherwise unproductive spawning and rearing area for salmon production. Clay (1961) described the design and use of fishways at natural obstructions and dams. Denil-type steeppass fishways up to 20.1 m in length have been used in the Columbia River Basin for Pacific salmon as well as some nonsalmonid fishes (Slatick and Basham 1985). Numerous studies of fishway performance have been conducted under laboratory conditions (Elling and Raymond 1959; Weaver 1962, 1967, 1969; Thompson and Gauley 1965; Thompson 1970; Slatick 1975; Katopodis and Rajaratnam 1983). Other investigators have studied the physiology and behavior of salmonids during migration and fishway passage (Connor et al. 1964; Ellis 1966; Smith et al. 1967; Brett 1973).

This paper contributes new information on the development and improvement of an Alaska steeppass fishway at Frazer Lake, Kodiak Island, Alaska, for passage of a major run of sockeye salmon (*Oncorhynchus nerka*). The experience gained from testing experimental modifications to improve salmon entry and passage at the Frazer fishway is of value and benefit in the design and construction of other similar fishways for salmon enhancement.

There are nine Alaska steeppass fishways that perpetuate introduced and native runs of salmon at six locations on Afognak and Kodiak Islands, Alaska. The most successful introduction of sockeye salmon on the Pacific Coast is at Frazer Lake (Blackett 1979). An impassable 10-m-high falls blocked passage of salmon into Frazer Lake until construction in 1962 of an Alaska steeppass fishway, described by Ziemer (1962). This fishway was still experimental at the time and inadequate by 1970 as the number of sockeye and chinook salmon (*O. tshawytscha*) annually returning to the fishway increased. Experimental modifications and improvements to the fishway were accomplished from 1971 through 1976 to increase salmon entry and passage and to develop a design for construction of

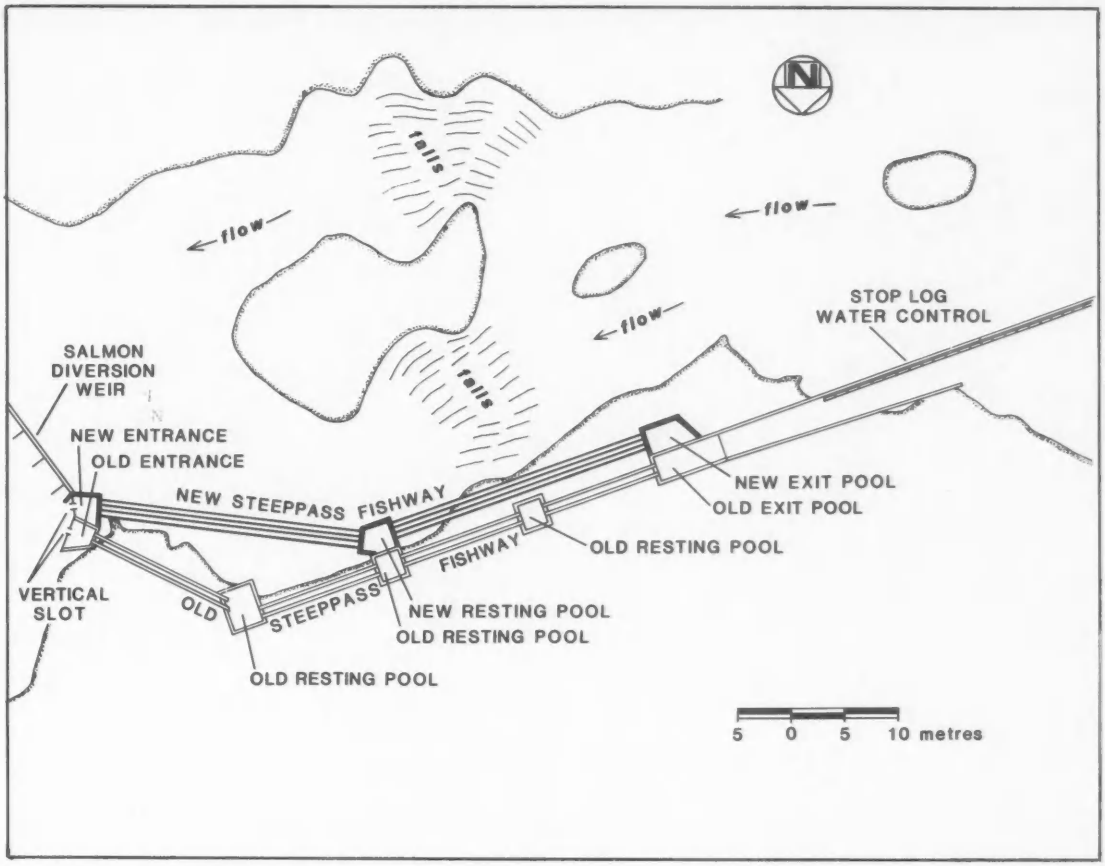

FIG. 1. Schematic drawing of the Frazer fishway after improvements to the original structure with three resting pools and addition of the new steeppasses (river side). Salmon counts were at the vertical slot entrance and the exit pools (top of falls).

adjoining steeppasses. A minimal requirement, after reconstruction, was the capability to pass 40 000 salmon a day and 400 000 salmon a year. After completion in 1979, fishway performance was evaluated through 1982. The present fishway (Fig. 1 and 2) with 68 sections of Alaska steeppass and eight tanks (entrance, resting, and exit) is probably the largest facility of its kind on the Pacific Coast and perhaps the world.

The objectives of fishway modifications were to (1) compare passage and exit rates of sockeye salmon in a 60-m-long steeppass fishway with resting pools with one without resting pools, (2) determine if resting tanks are necessary and beneficial, and (3) determine if the rate of salmon entry into the steeppasses could be improved by creating a nonturbulent stream for salmon attraction within the entry steeppasses. Evaluation of the modifications consisted of a 4-yr (1973–76) preconstruction study. Postconstruction evaluation was for 4 yr (1979–82) after completion of fishway reconstruction and expansion.

Fishway and Methods

The fishway is located (154°04′W, 57°13′N) at a falls 1.6 km downstream from Frazer Lake and 9.6 km from Olga Bay on the southwestern end of Kodiak Island in the Kodiak National Wildlife Refuge. Salmon ascending the river must negotiate a stretch of turbulent "white water" and a passable lower falls before reaching the fishway. The introduced run of sockeye salmon (target escapement of 383 000 (Blackett 1979)) is the primary species at Frazer Lake. Donor sources for the introduction, described in detail by Blackett (1979), were primarily from nearby Karluk and Red Lake sockeye stocks. Other salmon using the fishway include an odd-year run of about 10 000 pink salmon (*O. gorbuscha*) and lesser numbers of chum salmon (*O. keta*) and introduced chinook salmon.

In 1971 and 1972, salmon entry and passage rates were greatly improved by extending the fishway entrance downstream from the base of the falls, construction of a weir to guide all salmon migrating upstream to the entrance tank, construction of an entrance tank with a vertical slot opening, and altering water flow and velocity to attract salmon along the weir and into the entrance tank. Experimental modifications and evaluation were begun in 1973 to further improve performance and develop a design for construction of an additional steeppass alongside and parallel to the existing structure.

The Frazer fishway, before experimental modification in 1973, consisted of a U-shaped concrete entrance tank (3 m ×

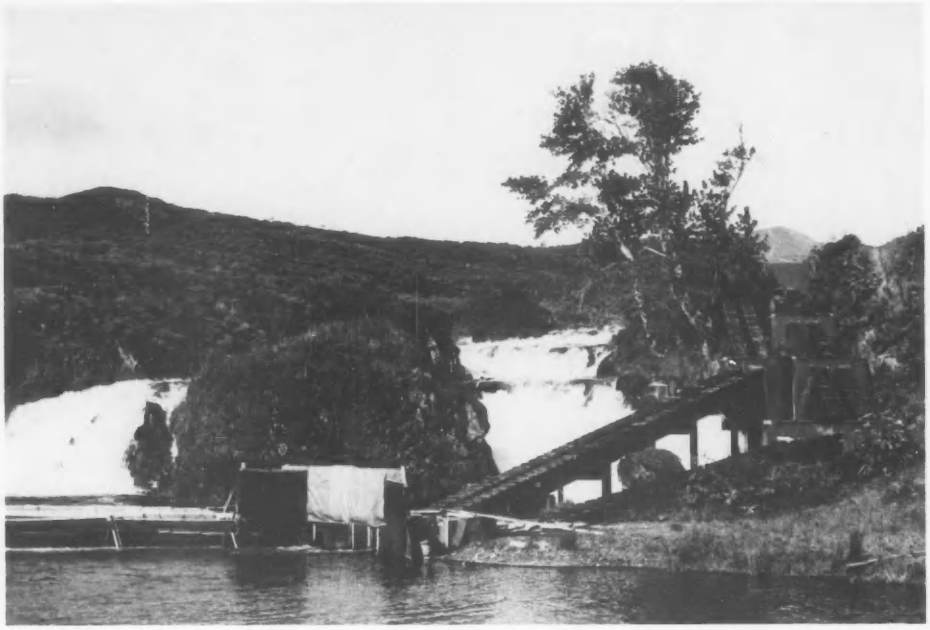

FIG. 2. Frazer fishway after construction of new steeppasses (left of original fishway), salmon diversion weir, and padded vertical slot entrance tank.

3 m × 1.5 m deep) downstream from the falls, two 15-m lengths of parallel type "A" steeppass sections from the entrance tank to a concrete resting tank (3 m × 4 m × 3 m deep), two more 12-m lengths of parallel steeppass, type "A" on one side and type "C" on the other side, to a second concrete resting tank (3 m × 3 m × 3 m deep), another 12-m length of parallel, type "A" and "C" steeppasses (side-by-side), to a third concrete resting tank the same size as the second tank, and a final 12-m length of paired type "A" and "C" steeppasses to a 3 m × 6 m × 2.5 m deep concrete exit tank at the top of the falls. The steeppasses are inclined at a slope of 22%. The type "A" and "C" Alaska steeppasses, modifications of the Denil fishway design, were developed by Ziemer (1962) for installation in remote sites of Alaska. Each steeppass section is 3.05 m long and consists of two aluminum sides 0.70 m deep and a bottom (0.56 m wide) that can be transported in amphibious aircraft and assembled on site. The type "A" steeppass inner vanes are perpendicular to the bottom. Type "C" steeppass vanes are angled (45°) upstream from the bottom. Katopodis and Rajaratnam (1983) described Denil fishways and the Alaska steeppass in detail. The type "A" steeppass has a lower (0.3 m/s) velocity but greater turbulence than the type "C".

An aluminum conduit and steel weir that extends 55 m from the entrance tank downstream at a 40° angle to the far bank guides all salmon migrating upstream to the fishway entrance. Fish enter the entrance tank through an adjustable slot opening. A narrow 22-cm slot opening provides increased water velocity for fish attraction until late in the migration when the opening is widened to 30 cm to reduce water velocity for older and less vigorous fish. Water depth in the steeppasses (operated 70–80% full) is controlled by a stop-log weir extending upstream from the fish exit tank at the top of the falls. Water depth in the entrance tank is maintained at approximately 1.2 m to submerge the steeppass ends.

In 1973, the fishway was modified by installing plywood extensions connecting the steeppass ends of a single run in each of the three resting tanks (Fig. 3). The interconnected steeppasses created a continuous 60-m length without any resting pools. The other steeppass run of the same length was left unchanged with the three resting pools. The fishway was further modified in 1974 by installing identical 1.8-m-long plywood chutes into each of the steeppass ends at the entrance tank. The chutes, tapered at the fish entry end to a 22-cm opening, created a less turbulent water stream for attraction of salmon from the entrance tank into the steeppasses. Plexiglas windows were installed in the top of each steeppass at the entrance tank for viewing salmon as they ascended the chutes and to provide additional light for attraction.

Observations

Sockeye salmon entrance, passage, and exit rates were evaluated by 15-min timed counts of the number of salmon entering the vertical slot entrance and exiting each of the steeppasses at the top exit tank during four periods each day: morning (09:00–11:30), midday (11:31–16:00), afternoon (16:01–19:00), and evening (19:01–23:00). Salmon exiting the modified steeppass without resting pools were counted either immediately before or after counting salmon exiting the steeppass with three resting pools. An observer counted at the entrance at the same time as another observer counted at the exit. In addition to timed counts, all salmon exiting the fishway were counted daily. It was intended that timed counts also be taken at each of the Plexiglas windows as fish entered the individual

FIG. 3. Fishway resting tanks showing temporary extensions that created a 60-m-long steeppass next to the unmodified steeppass. The lowermost resting tank still has a section of steeppass (now removed) that extended in the opposite direction to the original entrance at the base of the falls.

steeppasses. Accurate counting, however, was impossible at this location because of water turbulence and poor visibility. Counts of salmon exiting each steeppass were analyzed by time period and the difference in counts between the paired steeppass runs (modified and unmodified) tested by Student's *t*-test (Simpson et al. 1960) for the difference between the means.

Results and Discussion

Sockeye Passage in Modified and Unmodified Steeppasses

Fish passage, based upon the exit counts, was greater in the steeppass with resting pools for every time period during the peak of migration (June 21 – July 25) in 1973 (Table 1). However, salmon passage was only significantly greater during the evening period ($P < 0.02$). Greater passage in the evening was expected because salmon accumulate in the resting pools during the day and complete passage in the evening after entry rates decline.

Salmon were counted in the latter part of the run in 1973 to determine if older fish, with a greater liklihood of fatigue, would have decreased ability to ascend the steeppass without resting pools. By this time, 81% of the 56 255 sockeye salmon return had exited the fishway. Surprisingly, more salmon used the modified steeppass in the morning, afternoon, and evening during this latter counting period, but the difference in counts between steeppass runs was insignificant ($P > 0.05$). Overall, the greater number of fish using the steeppass with resting pools (55.9% of the 2872 salmon counted in 1973) was significant ($P < 0.02$).

In 1974, 55.6% of 3567 salmon counted (one hundred and sixteen 15-min periods) used the modified steeppass run without resting pools. The difference in passage was significant for the morning and evening periods and for all periods combined for the year (Table 1). In 1974, counts were only taken during the peak period (94% of total passage) of the 82 609 salmon return.

Unexpected year-to-year differences in steeppass use in this experiment could have resulted from a greater number of sock-

TABLE 1. Sockeye salmon passage (number exiting either steeppass in 15 min) in modified and unmodified steeppass fishways, Frazer Falls, 1973–76. *Significant at $P < 0.02$; other values nonsignificant at $P > 0.05$.

	Modified steeppass (no resting pools)				Unmodified steeppass (3 resting pools)				
Period	No.	%	$\bar{x}$	No. of observations	No.	%	$\bar{x}$	No. of observations	t-value
June 21 – July 5, 1973									
Morning	196	43.8	14.0	14	251	56.2	17.9	14	1.7133
Midday	235	45.9	18.1	13	246	51.1	18.9	13	0.3572
Afternoon	263	44.6	20.2	13	327	55.4	25.2	13	1.3036
Evening	352	37.3	20.7	17	592	62.7	34.8	17	2.4136*
July 6 – 26, 1973									
Morning	39	56.5	1.9	21	30	43.5	1.4	21	0.9100
Midday	25	46.3	1.5	17	29	53.7	1.7	17	0.4159
Afternoon	68	55.7	3.8	18	54	44.3	3.0	18	1.4748
Evening	88	53.3	5.9	15	77	46.7	5.1	15	0.8826
Total	1266	44.1	9.9	128	1606	55.9	12.5	128	2.6121*
June 7 – July 7, 1974									
Morning	394	59.2	14.6	27	271	40.8	10.0	27	2.8072*
Midday	428	50.5	14.8	29	419	49.5	14.4	29	0.1476
Afternoon	565	55.1	20.9	27	460	44.9	17.0	27	1.6040
Evening	595	57.8	18.0	33	435	42.2	13.2	33	4.2319*
Total	1982	55.6	17.1	116	1585	44.4	13.7	116	3.7265*
June 16 – July 20, 1975									
Morning	243	37.9	7.8	31	399	62.1	12.9	31	4.1907*
Midday	282	41.0	10.1	28	406	59.0	14.5	28	3.3689*
Afternoon	587	43.5	19.6	31	763	56.5	25.4	31	2.9589*
Evening	514	46.4	16.1	32	593	53.6	18.5	32	1.8237
Total	1626	42.9	13.3	122	2161	57.1	17.7	122	5.8000*
June 16 – July 19, 1976									
Morning	636	46.4	19.9	32	734	53.6	22.9	32	1.4978
Midday	711	54.7	22.9	31	589	45.3	19.0	31	1.0030
Afternoon	1126	56.9	35.2	32	853	43.1	26.6	32	1.2218
Evening	887	56.4	28.6	31	686	43.6	22.1	31	1.3009
Total	3360	54.0	26.7	126	2862	46.0	22.6	126	1.6350
Total 1973–76	8234	50.1	16.7	492	8214	49.9	16.7	492	0.0708

eye in 1974 than in 1973, the ascent of 1777 pink salmon in 1973, but the absence of this species in 1974, and more chinook salmon using the fishway in 1973. Chinook salmon will occasionally reside in the resting pools and go down the fishway. In the fishway without resting pools, descent of chinook salmon delays passage of other fish for a longer time than in the fishway with resting pools.

Evaluation of sockeye salmon passage was continued during the peak of migration in 1975 and 1976 (Table 1). In 1975, the unmodified steeppass was favored (57.1% of fish passage) with a highly significant ($P < 0.01$) difference in passage for the year. In 1976, passage was greater in the modified steeppass (54.0% of the fish), but the difference in passage was nonsignificant ($P > 0.05$) for all time periods and for the year.

Over the 4-yr study (1973–76), the unmodified steeppass had greater passage of sockeye salmon in odd years (1973 and 1975) and the modified steeppass had greater passage in even years (1974 and 1976). For all years and time periods combined, sockeye salmon passage was nearly equivalent (~50% each steeppass) in modified and unmodified steeppasses (Table 1), but significant year-to-year differences in salmon passage occurred between the steeppasses.

Results indicate that (1) sockeye salmon (also pink, chum, and chinook salmon that were observed, but not specifically studied) were capable of ascending a continuous steeppass fishway of 60 m length and 22% slope without visible fatigue or resting, (2) resting tanks at intervals between long steeppass runs can be beneficial in providing a holding area for slow moving or descending fish without blocking passage of other fish and increases overall capacity of the fishway, (3) salmon passage in a fishway with resting tanks may be delayed because of salmon holding in the tanks, (4) there was no overall conclusive advantage for salmon passage in a fishway either with or without resting tanks, and (5) there can be considerable variation from year to year in sockeye salmon passage in the fishway. Greater use of the fishway by other salmon species in some years and variable year-to-year environmental conditions (water flow, velocity, and temperature of the river) quite likely affect the physical capability of sockeye salmon to ascend the fishway.

TABLE 2. Evaluation of sockeye salmon entry (based upon 15-min counts periodically each day) into the Frazer fishway by year, 1973–76.

Period	No. of 15-min observations	Entrance structure: Fish in: No.	Fish in: Average per 15 min	Fish in: Range per 15 min	Fish out[a]: No.	Fish out[a]: Average per 15 min	Fish out[a]: Range per 15 min	Fish out[a]: % dropout[b]
June 24 – July 26, 1973	117	7 566	64.7	0–282	3023	25.8	0–134	40.0
June 7 – July 7, 1974	109	5 607	51.4	0–315	718	6.6	0–34	12.8
June 16 – July 20, 1975	131	8 984	68.6	1–266	2719	20.7	0–82	30.3
June 16 – July 19, 1976	126	11 372	90.2	0–271	3163	25.1	0–93	27.8
Total	483	33 529	69.4	0–315	9623	19.9	0–134	28.7

[a]Number of fish dropping out of the entrance instead of ascending the steeppass.
[b]Number of fish dropping out expressed as a percentage of fish entering the entrance structure.

TABLE 3. Evaluation of sockeye salmon entry (based upon 15-min counts periodically each day) into the Frazer fishway entrance by time period, 1973–76.

Observation period	No. of 15-min observations	Entrance structure: Fish in: No.	Fish in: Average per 15 min	Fish in: Range per 15 min	Fish out[a]: No.	Fish out[a]: Average per 15 min	Fish out[a]: Range per 15 min	Fish out[a]: % dropout[b]
Morning	123	7 445	60.5	0–257	2083	16.9	0–114	28.0
Midday	117	8 167	69.8	0–282	2466	21.1	0–134	30.2
Afternoon	118	9 169	77.7	0–271	2823	23.9	0–120	30.8
Evening	125	8 748	70.0	0–315	2251	18.0	0–117	25.7
Total	483	33 529	69.4	0–315	9623	19.9	0–134	28.7

[a]Number of fish dropping out of the entrance instead of ascending the steeppass.
[b]Number of fish dropping out expressed as a percentage of fish entering the entrance structure.

The new fishway (Fig. 1), based upon these results, had a single resting tank incorporated midway between the entrance and exit tanks. Steeppass runs entering and exiting this tank were approximately 24 m long. The single resting tank was a compromise between the advantages and disadvantages of resting tanks even though sockeye salmon were proven capable of a continuous 60-m-long ascent without resting.

Sockeye Entry into the Vertical Slot Entrance

The average salmon entry count in 1973 was 64.7 in 15 min (117 observations), but an average of 25.8 salmon (39.9%) dropped out of the entrance instead of ascending the fishway during the same time. In 1974, plywood chutes were installed in the entry end of the steeppasses in an attempt to increase passage in the fishway and decrease fish dropout from the entrance. The chutes created a high-velocity water stream for salmon attraction similar to the vertical slot entrance and eliminated the turbulent circular water flow patterns caused by the steeppass vanes. After the chutes were installed the rate of salmon dropout decreased from 39.9% in 1973 to 12.8% in 1974 (Table 2). Initial results were encouraging, but in 1975 and 1976, dropout rate increased to 30.3 and 27.8%, respectively. Even at these higher rates, the chutes were considered partially effective in increasing salmon passage from the entrance tank into the fishway steeppasses, decreasing salmon dropout from the entrance tank.

Overall, sockeye salmon entry into the entrance averaged 69.4 fish (range 0–315) in 15 min for the 4-yr study (Table 2). Entry rate was typically rapid early in the run and decreased after the peak of migration. The average entry rate varied from 51.4 salmon/15 min in 1974 to 90.2 salmon/15 min in 1976. A relationship between salmon density below the fishway and the entry rate probably exists, to some point of maximum entry speed, but was not consistently apparent in this study. The average dropout was 19.9 fish in 15 min for the 4 yr (low of 6.6 fish in 1974 and high of 25.8 fish in 1973). Entrance dropout varied with entrance rate, salmon holding capacity of the entrance tank, and with the rate at which salmon ascended the steeppasses.

Afternoon was the peak sockeye salmon entry period (average of 77.7 fish in 15 min) and morning was the slowest period (average of 60.5 fish in 15 min) (Table 3). Salmon dropping out of the entrance ranged from 23.9 fish in 15 min in the afternoon to 16.9 fish in 15 min in the morning. The proportion of fish dropout (30.8%) was highest in the afternoon, which coincided with the peak period of fish entry, and lowest (25.7%) in the evening. A consistent pattern of sockeye salmon migrating upstream during daylight with increasingly greater intensity during the day, peaking by early evening, and ending during darkness (after 11:00 p.m.), typically occurs at Frazer. At night, sockeye salmon frequently move downstream. Peak passage and rapid upstream movement usually occurred on sunny days.

The vertical slot orifice of the fishway entrance tank is highly effective in attracting sockeye salmon into the entrance, but has the disadvantage of causing fish to jump rather than swim into the slot opening. Water passing through the restricted opening at 2.1–2.5 m/s creates a small fall with turbulent "white water" at the base. Sockeye salmon have a natural tendency to jump when these conditions exist and tend to jump far more

TABLE 4. Sockeye salmon passage before construction (1973–76) and after construction (1979–83) of the Frazer fishway.

Year	No./yr	No./h[a]	No./d	% of total passage per day	Peak passage date
			Preconstruction		
1973	56 255	768	6 822	12.1	June 23
1974	82 609	435	7 378	8.9	June 26
1975	64 199	448	5 436	8.5	July 8
1976	119 321	872	9 186	7.7	June 29
			Postconstruction		
1979	126 742	984	8 889	7.0	June 28
1980	405 525	2784	38 058	9.4	June 30
1981	377 716	3044	31 109	8.2	June 17
1982	437 772	2160	34 553	7.9	July 12
1983	158 340	—	12 776	8.1	July 1

[a]Expansion of maximum 15-min exit count.

frequently than pink, chum, or chinook salmon. A padded backboard at the entrance prevents injury of jumping fish. An experimental modification that placed the slot opening underwater to decrease jumping was unsuccessful because attraction and entry rate decreased to unacceptable levels. A change of a few centimetres in location and size of the vertical slot opening substantially decreases water depth in the tank, velocity of water outflow, and the attraction necessary to maintain satisfactory sockeye salmon entry.

Sockeye Passage and Entry

Annual passage of sockeye salmon for 4 yr (1973–76) prior to construction of the additional fishway ranged from 56 255 to 119 321 fish (Table 4). The maximum rate of passage in 1976, based upon expansion of the 15-min exit count, was 872 sockeye salmon/h and 9186 salmon/d. The maximum passage rate in other years (1973–75) ranged from 435 to 768 salmon/h and 5436 to 6822 salmon/d. The peak daily passage was 7.7–12.1% of annual passage for all years.

A requirement for the fishway (400 000 sockeye annually and 40 000/d) upon completion of new construction meant capability for passage of up to 2500 salmon/h (625/h per steeppass) for a period of 16 h on a peak passage day. Passage rates of this magnitude require exceptional fishway performance.

The number of salmon completing passage in the fishway, during the timed counts, was only 68.8% (60.4–75.8%) of the net number of salmon calculated to be left in the entrance tank after dropout, overall for the 4-yr period (Table 5). The highest proportion of salmon in the entrance to exit the fishway occurred the same years (1974 and 1976) that salmon passage was greatest in the steeppass without resting pools. The lowest average entry rate in 1974 coincided with the second highest passage (82 609 sockeye) and the second highest passage in 3 d (19 028 sockeye). The timed entry and exit counts provide a relative comparison of entry and passage at a point in time, but not actual passage rates because a single salmon can be counted repeatedly entering and dropping out of the entrance and only once at the fishway exit.

The highest average entry rate and peak sockeye passage (119 321; 20 095 in 3 d) occurred in 1976. Salmon exit counts, like the entrance counts, were greatest in the afternoon and evening periods (Table 6). Salmon passage, expressed as a percentage of those fish in the entrance at a given time, increased substantially after midday as the density of salmon increased in the entrance. The unmodified steeppass had 3.0% more fish exiting in the morning period and 0.8–2.4% less fish for the other periods than the modified steeppass. Accummulation of salmon in the resting pools of the unmodified steeppass could have resulted in lower passage rates during the day than the steeppass without resting tanks.

The primary factor limiting salmon passage at the Frazer fishway is the ability to get salmon from the entrance tank into the steeppasses. Passage rates can be improved by decreasing entrance dropout and increasing salmon entry into the steeppasses at approximately the same rate as salmon enter the vertical slot entrance.

Postconstruction Evaluation

Construction of the new fishway and entrance tank, enlargement of the exit tank, and extension of the water control structure were completed in the spring of 1979. The temporary plywood steeppass extensions in the resting tanks of the original fishway (creating a continuous steeppass) were removed. The plywood chutes in the steeppass ends at the fish entrance tank were left in place in the original fishway, but not installed in the steeppasses of the new fishway. The new fishway entrance adjoined the original entrance, the single resting tank of the new fishway adjoined the second resting tank of the original fishway about midway to the top, and the new exit tank adjoined and opened into the original exit tank (Fig. 1). The exit tank at the top of the falls was doubled in length which, combined with the new tank, tripled salmon holding capacity to 2500–3000 fish. The water control weir was extended across the river to maintain optimal water supply by addition or removal of stop logs in thirty-five 1.2-m-wide sections.

The purpose of postconstruction evaluation was (1) to determine overall fishway performance, (2) to determine success of the new fishway compared with the original fishway, and (3) to evaluate design and construction of the new fishway, based upon the results of earlier evaluation. When postconstruction evaluation began in 1979, the fishway consisted of the original two runs of steeppasses interconnected with three resting tanks and two new runs of type "A" steeppass with one resting tank midway between the entrance and exit tanks (Fig. 1 and 2). Timed counts of salmon in the original and new fishway addition were continued at the entrance tanks through 1981 and the exit tank through 1982.

Sockeye salmon passage ranged from 126 742 in 1979 to 437 772 in 1982 (Table 4). Peak rates of salmon passage of 3044/h in 1981 and 38 058/d in 1980 suggested that design criteria for passage of 40 000 salmon/d and 400 000/yr was achievable. (In fact, as this paper was being written in 1985, a new daily passage record of 46 805 sockeye salmon in 16 h occurred on July 1.) The daily peak passage in 1980 would have exceeded 40 000 had there been sufficient salmon below the fishway.

The additional passage capability provided by the new fishway, although sufficient to achieve overall passage requirements, was substantially less than the old fishway. Salmon passage from 1979 to 1982 was 31–69% greater in the original (old) fishway (Table 7) than in the new fishway. The higher rate of passage in the old fishway was consistent for all time periods and all years. The highest average count of sockeye

TABLE 5. Sockeye salmon passage (based upon 15-min counts periodically each day) compared with number of salmon entering the entrance structure, Frazer fishway, 1973–76.[a]

Year	Adjusted no. of fish in entrance[a]	Unmodified steeppass (three resting pools)		Modified steeppass (no resting pools)		Total	
		No. of fish	% of fish in entrance	No. of fish	% of fish in entrance	No. of fish	% of fish in entrance
1973	4 543	1606	35.3	1266	27.9	2 872	63.2
1974	4 889	1585	32.4	1982	40.5	3 567	72.9
1975	6 265	2161	34.5	1626	25.9	3 787	60.4
1976	8 209	2862	34.9	3360	40.9	6 222	75.8
Total	23 906	8214	34.4	8234	34.4	16 448	68.8

[a]Number of salmon in the entrance structure after dropouts (fish entry count minus the fish dropout).

TABLE 6. Sockeye salmon passage (based upon 15-min counts periodically each day) compared with number of salmon entering the entrance structure by time of day, Frazer fishway, 1973–76.

Observation period	No. of fish in entrance[a]	Unmodified steeppass (three resting pools)		Modified steeppass (no resting pools)		Total	
		No. of fish	% of fish entering	No. of fish	% of fish entering	No. of fish	% of fish entering
Morning	5 362	1685	31.1	1508	28.1	3 193	59.5
Midday	5 701	1689	28.6	1681	29.5	3 370	59.1
Afternoon	6 346	2457	38.7	2609	41.1	5 066	79.8
Evening	6 497	2383	36.7	2436	37.5	4 819	74.2
Total	23 906	8214	33.4	8234	34.4	16 448	68.8

[a]Number of fish entering minus the number of fish dropping back out of entrance structure.

salmon exiting the facility in 15 min was 314.3 and 116.0 during the afternoon in 1981 for the old and new fishways, respectively.

Sockeye salmon entered the new fishway entrance in greater numbers in 1979 and into the old fishway entrance in greater numbers in 1980 and 1981 (Table 8). The average entry rate in 15 min varied from 64.6 fish in 1979 to 142.0 fish in 1980 in the old fishway entrance. The lowest and highest average entry rates for the new fishway entrance were 90.5 and 124.3 fish in 15 min. Overall, 16.4% more fish entered the new fishway entrance in 1979, 6.6% more fish entered the old entrance in 1980, and 5.8% more fish entered the old entrance in 1981.

The rate at which sockeye salmon dropped out of the entrance structures was greater for the new entrance by 3.2% in 1979, 41.5% in 1980, and 17.9% in 1981. Fish dropout (Table 8) ranged from 11.6 to 34.5% of fish entering for the old entrance and from 29.5 to 54.7% for the new entrance. The net fish entry (entry count minus dropout count in 15 min) ranged from a yearly average of 56.3 (1979 and 1980) to 78.8 for the new entrance and from 42.3 to 110.9 for the old entrance. Overall, net fish entry, based upon the timed counts, was greater in the new entrance in 1979 by 13.8% and in the old entrance by 37.2% in 1980 and by 16.8% in 1981. The lower net fish entry in the new entrance in 1980 and 1981 could have been a reason for lower exit rates in the new fishway in those years, but not in 1979. In 1979, the new fishway had a far lower average exit count (23.3 fish less), but an average net fish entry that was 14.0 fish greater during the timed counts than the old fishway. Higher dropout and lower exit counts suggest more salmon falling back rather than ascending the new fishway addition than in the original fishway.

Overall, the new fishway had poorer performance in fish entry in 2 of the 3 yr studied and in salmon passage and exit rates in all years; however, the new fishway sufficiently increased salmon passage capability to meet minimum passage goals. The better performance of the old fishway in net fish entry and salmon passage could be due in part to the plywood chutes left in the entry steeppasses of the old fishway, but not installed in the new fishway.

General Discussion

Salmon passage studies at Frazer demonstrate that sockeye salmon have the capability of ascending long steeppass runs of moderate slope (60 m in length and 22% slope) without intermediate resting pools. However, resting pools were advantageous in increasing system capacity and providing a holding area for slower fish that block the steeppass. Postconstruction evaluation showed that the new fishway constructed with a single midway resting tank was less effective with considerably lower salmon passage than the original fishway with three intermediate resting tanks. Therefore, the benefits of resting tanks may be greater than the disadvantages of additional construction cost and short-term delay of fish that rest. Ellis (1966) found that in fast current (>0.5 m/s), sockeye salmon swimming behavior is to dart and then hold position at intervals. A fishway with resting tanks may be better suited to the normal swimming behavior of sockeye salmon than a long fishway without resting tanks. Paulik et al. (1957) found coho salmon (*O. kisutch*) to be highly susceptible to fatigue in swimming performance tests and concluded that adequate resting facilities are necessary in fishways with water velocities exceeding

TABLE 7. Sockeye salmon passage (based upon 15-min counts of salmon exiting the fishways) in old and new steeppass fishways, Frazer Falls, 1979–82.

Period	Old steeppasses				New steeppasses			
	No.	%	$\bar{x}$	No. of observations	No.	%	$\bar{x}$	No. of observations
June 8 – July 12, 1979								
Morning	1 274	69.2	38.6	33	556	30.4	18.5	30
Midday	1 655	62.9	44.7	37	977	37.1	25.1	39
Afternoon	1 832	65.8	59.1	31	953	34.2	31.8	30
Evening	1 840	65.0	57.5	32	991	35.0	30.0	33
Total	6 601	65.5	49.6	133	3477	34.5	26.3	132
June 22 – July 23, 1980								
Morning	2 764	81.3	106.3	26	637	18.7	24.5	26
Midday	4 629	84.6	171.4	27	841	15.4	31.1	27
Afternoon	3 529	84.4	235.3	15	651	15.6	43.4	15
Evening	3 950	86.9	188.1	21	594	13.1	28.3	21
Total	14 872	84.5	167.1	89	2723	15.5	30.6	89
June 21 – July 1, 1981								
Morning	1 516	61.8	126.3	12	936	38.2	78.0	12
Midday	2 613	73.3	239.2	11	950	26.7	86.4	11
Afternoon	943	73.0	314.3	3	348	27.0	116.0	3
Evening	1 280	74.5	256.0	5	437	25.5	87.4	5
Total	6 352	70.4	204.9	31	2671	29.6	86.2	31
July 10 – 13, 1982								
Morning	1 078	81.3	269.5	4	248	18.7	62.0	4
Midday	1 429	84.1	285.8	5	270	15.9	54.0	5
Afternoon	772	80.2	257.3	3	190	19.8	63.3	3
Evening	693	85.3	231.0	3	119	14.7	39.7	3
Total	3 972	82.8	264.8	15	827	17.2	55.1	15

TABLE 8. Sockeye salmon entry (based upon 15-min counts of salmon entering and exiting the entrances) into old and new steeppass fishway entrances, Frazer Falls, 1979–81.

Fishway entrance	No. of observations	Fishway entry			Fish dropout				Net fish entry		
		No.	$\bar{x}$	Range	No.	$\bar{x}$	Range	% dropout[a]	No.	$\bar{x}$	Range
June 8 – July 12, 1979											
Old	133	8 588	64.6	0–252	2962	22.3	0–92	34.5	5 626	42.3	−29–198
New	132	11 941	90.5	0–246	4507	34.1	0–111	37.7	7 434	56.3	−23–208
June 22 – July 23, 1980											
Old	89	12 639	142.0	10–473	1668	18.7	1–115	13.2	10 971	123.3	7–358
New	89	11 060	124.3	9–359	6045	67.9	2–290	54.7	5 015	56.3	−12–223
June 12 – July 1, 1981											
Old	31	3 888	125.4	8–245	451	14.5	1–35	11.6	3 437	110.9	7–217
New	31	3 468	111.9	43–210	1024	33.0	1–97	29.5	2 444	78.8	37–141

[a]Number of fish dropping out expressed as a percentage of fish entering the entrances.

1.1 m/s for any considerable distance. They felt that to prevent exhaustion, salmon should not be forced to maintain a strenuous effort for more than a few minutes.

The poorer performance of the new fishway could be due, in part, to unplanned changes that occurred in construction. Elevation of the new steeppasses at the exit tank (top of falls) was slightly higher than the old steeppasses. Adequate water flow in the new fishway requires slightly higher flow in the old fishway. This additional flow might have attracted more sockeye salmon into the old fishway. Additionally, the high-velocity slot opening in the new fishway entrance was slightly larger than the slot opening in the other entrance, which combined with lower flow, further decreased attraction velocity at the new entrance. Less attraction flow at the new entrance, however, did not result in an entry rate lower than the old entrance in 1979, but may have been a factor in 1980 and 1981

when entry rate was lower. Improvements to increase flow and attraction in the new fishway are planned to increase capability for more salmon to ascend the fishway in less time and with less effort and energy expenditure.

Timed counts at the Frazer fishway entrances varied in accuracy. Observations were sometimes affected by the number of fish entering and exiting at one time and water turbidity. Counts known to be inaccurate were omitted from analysis, and could have created some bias, especially for peak entry time periods. All exit counts were considered accurate and used in the analyses.

Special attention has been given to the Krazer fishway because of the highly successful sockeye salmon introduction, necessity for passage of up to 400 000 sockeye salmon annually over the unpassable falls, the short peak migration period (<4 wk), and the height (10 m) and width (~30 m) of the fall the fishway bypasses. Experimental modifications of the original (1962) fishway were essential to solve poor entry and delayed passage problems that occurred as the sockeye returns progressively increased in the 1960s and 1970s. There were three major problems with the fishway.

(1) Sockeye salmon were attracted to the greater flow over the falls rather than the relatively minor flow from the fishway entrance orifice at the base and to one side of the waterfall. Salmon entry was so poor that less than half of the returning sockeye in the late 1960s, when escapements were still minor (<30 000 fish/yr), ascended the fishway after 2 or more wk. By 1969, the problem was so acute that 5000 salmon had to be seined from the river below the falls and manually placed in the fishway. Extension of the steeppasses, construction of the new fishway entrance 33 m downstream of the falls, and installation of the weir to divert and guide all salmon away from the falls to the entrance solved the problem of attraction to the fishway.

(2) The U-shaped entrance configuration, constructed in 1973, proved ineffective because salmon accumulated in front of the entrance, but failed to move upstream into the steeppasses. Reconstruction of the entrance to form the enclosed tank with a vertical slot opening (width adjustable from 22 to 30 cm) was highly successful for attracting salmon into the fishway entrance. Considerable experimentation with the size and location of the slot opening resulted in an optimal orifice size (22 cm wide × 28 cm long) beginning 56 cm above the tank bottom. This size orifice provided the necessary velocity (2.1−2.5 m/s) and head differential between water in the tank and river surface to attract sockeye salmon into the entrance at a rapid rate.

(3) Entry rate of sockeye salmon into the steeppasses was less rapid than into the entrance tank. Salmon dropped out of the tank instead of going up the steeppasses when tank capacity was exceeded. Chutes installed in the entry ends of the steeppasses in 1974 created better flow and velocity for salmon attraction and were partially successful in increasing passage and decreasing dropout.

The passage capability of most fishways is limited by ability to attract salmon to the entrance, entry rate into the entrance structure, and the holding capacity of the entrance tank or pool. The experience gained from the trial modifications at Frazer suggests that improved engineering design of entrance tank configurations, entrance orifices, entry steeppass sections, and entrance tank holding capacity can greatly increase fishway efficiency.

At the Frazer fishway, the efficiency is judged by the ratio of fish successfully achieving passage to total fish escapement and the rate of fish passage. The goal is to achieve at least 95% passage of the salmon escapement over the falls with a delay of less than 24 h. Delayed salmon passage and excessive energy expended in passage can decrease ability to reach spawning areas and ultimately spawning success. Black (1958) found that severe muscular activity from swimming through swift water can be lethal in salmon. Paulik and DeLacy (1958) found a definite decline in swimming ability of Columbia River sockeye as they migrated upstream to the spawning area. They suggest that salmon motivated to move rapidly in a fishway may become so fatigued that a long rest period in some other part of the fishway is required to recover from the exertion. Recovery from exhaustion was thought to take longer the further upstream the salmon had progressed. Construction of fishways that avoid high energy expenditure and stress is therefore essential to minimize fish loss.

The proper location of fishway entrances and provision of sufficient water for salmon attraction and rapid passage is also critical. Each species of salmon has been observed to have unique requirements for water flow and velocity for optimal entry and passage as well as specific behavior patterns in the fishway. The distinct preferences of each salmon species and species-specific capabilities for fishway ascent have also been demonstrated under conditions of optional water flows, velocities, and temperatures at the Bonneville Dam Laboratory (Weaver 1962, 1967, 1969; Slatick 1975; Thompson 1970). Flow requirements frequently change during the migration period and from year to year requiring flexibility, such as at Frazer, in fishway operation. The water velocity required to attract sockeye salmon, for example, can often be too great for optimal passage of pink salmon. At other times, the physical condition of salmon and water quality (turbidity, temperature, and at times of low flow and warm water, dissolved oxygen) limits passage ability. Changes in the temperature, pH, and dissolved gases in the route of adult salmon in Russia were considered by Krogius (1959) to cause unequal intensity in the migration on adjacent days. Water quality influences metabolism and the energy required by salmon for migration, maturation, and spawning (Brett 1962; Banks 1969). Physical condition, as influenced by age, maturity stage, stress and fatigue, health, and probably to some extent the inherent genetic capability of the stock for upstream migration, affects passage rate and capability for ascent of fishways. Connor et al. (1964), in experiments with "endless" fishways, demonstrated a progressive expenditure of muscle glycogen in sockeye and chinook salmon in fishways of moderately high ascent. Black (1958) suggested that death occurs from the failure of an adequate reserve of glycogen. Adult sockeye salmon in the Fraser River (Canada) utilize up to 90% of the body fats and 50−60% of the total protein in migration to spawning area (Idler and Clemens 1959). Therefore, the energy an adult salmon must expend to reach and ascend a fishway and then finally to successfully reach spawning areas and spawn is an important consideration in fishway design and construction.

The variability from year to year in salmon entry and passage rates at the Frazer fishway is attributed, in part, to condition of fish and variable water quality, flow, and velocity. Sockeye salmon at Frazer must negotiate 9.6 km of river including a 6-km stretch of steep gradient and rapid velocity as well as a lower, passable fall before reaching the fishway. Seasonal variation in fish condition at Frazer (vigorous salmon early in the run to less vigorous as the run progresses) requires reduction of water velocity at the vertical slot entrance for passage of fish

late in the season.

Observations of rapid movement of sockeye salmon and increased entry and passage at the Frazer fishway when sunlight is intense confirm the importance of light as an attractant in fishways. When sockeye salmon were given a choice of entering lighted and darkened submerged orifices with equivalent flows at the Bonneville Laboratory, over 90% of the fish selected the lighted orifice (Weaver 1967). Krogius (1959) concluded that the intensity of light has only an incidental significance in migration of adult sockeye salmon in Russia because the runs occur both at night and under conditions of the brightest light.

Sockeye salmon at Frazer travel from a weir at the river mouth (Olga Bay) to the fishway in approximately 3–7 d. Negotiation of the fishway and upper river to Frazer Lake usually takes less than 1 d. The migration rate of ~1.9 km/d at Frazer, based upon a 6-d median, is less than the 3.4–4.8 km/d determined by Gard (1973) for sockeye migration up Karluk River, 19.0 km/d for sockeye to migrate the Snake River (Thompson et al. 1958), and 10 km/d for sockeye in the Columbia River (Schoning et al. 1950). The slower travel time for Frazer sockeye is attributed primarily to the turbulent flow, high velocity, and lower falls downstream of the fishway. Ellis (1966), in testing sockeye salmon swimming speeds, found that water flows of high velocity, like the lower river at Frazer, resulted in position holding after periods of high-speed (1.0–1.4 m/s) dart swimming. The need for extensive position holding or resting tends to increase migration travel time.

The Frazer fishway, with passage capability of over 2900 sockeye salmon/h, is believed to be the largest steeppass structure on the Pacific Coast. The Frazer Lake run, perpetuated by the fishway, could return over 0.7 million sockeye salmon a year (Blackett 1979) and contributes fish to major seine and set gillnet fisheries. The experience gained from the problems and solutions, experimental modifications, and performance of the Frazer fishway should be of substantial value in the design, construction, and operation of other fishways for salmon enchancement.

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Food Quality and Hydropsychid Caddisfly Density in a Lake Outlet Stream in Glacier National Park, Montana, USA

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Valett, H. M., and J. A. Stanford. 1987. Food quality and hydropsychid caddisfly density in a lake outlet stream in Glacier National Park, Montana, USA. Can. J. Fish. Aquat. Sci. 44: 77–82.

Hydropsychid caddisfly larval density averaged 6500/m^2 in the lake outlet, but decreased to 1427 and 209/m^2 at a distance of 200 and 3400 m downstream. Food quantity, measured as the concentration of particulate organic carbon in grab samples of seston, differed significantly between sites on only 3 of 13 sampling dates. But food quality variables (i.e. protein and ATP content of seston) were significantly higher in the lake outlet during months of higher lake productivity; monthly values of particulate protein regressed negatively with distance downstream. We concluded that stable substrate, discharge, and temperature regimes, combined with a plentiful supply of high-quality food, maintained high densities of filter-feeding hydropsychids in the lake outlet. Downstream populations were limited by comparatively greater abiotic variation (i.e. discharge, substratum, diel temperature) and by a concomitant decrease in seston food quality.

La densité larvaire de phryganes était de 6 500/m^2, en moyenne, à l'exutoire du lac, mais tombait à 1 427/m^2 et 209/m^2 à 200 et 3 400 m en aval. La quantité de nourriture, mesurée en concentration de carbone organique sous forme de particules dans des échantillons pris au hasard dans le seston, différait de manière significative entre les stations dans seulement 3 des 13 dates d'échantillonnage. Cependant, les variables de qualité alimentaire (c.-à-d. la teneur en protéines et en ATP du seston) étaient significativement plus élevées à l'exutoire du lac durant les mois de productivité intense du lac; les concentrations mensuelles des protéines en particules diminuaient avec la distance en aval. Nous sommes arrivés à la conclusion qu'un substrat stable et les régimes thermiques et d'écoulement de l'eau, combinés à une réserve alimentaire abondante et de haute qualité, assurent le maintien d'une densité élevée de phryganes, organismes filtrants, dans l'exutoire du lac. Les populations en aval étaient limitées par des variations abiotiques comparativement plus importantes (c.-à-d. le déversement, le substrat, la température diel) et par un abaissement concomitant de la qualité alimentaire du seston.

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Larvae of filter-feeding aquatic insects are often very abundant in lake outlet streams (Ulfstrand 1968; Carlsson et al. 1977; Wotton 1978; Brönmark and Malmqvist 1984). However, population density often decreases dramatically as one moves downstream from the lake (Statzner 1978; Oswood 1979), perhaps in response to a gradient in the quantity and/or quality of food resources.

Since seston (i.e. particles transported in the water column of the stream) is generally thought to comprise the forage base of most filter-feeding insects living in lotic systems (Wallace et al. 1977; Colbo and Porter 1979; McCullough et al. 1979; Wallace and Merrit 1980), it may be that large quantities of high-quality sestonic foods may support many filter-feeders in the outlet. But biotic processing of lake-derived seston in the outlet area, coupled with introduction of fluvial porcesses, may progressively deplete the quantity or somehow alter the quality of the resources available to filter-feeders living in downstream reaches of the same stream (Maciolek and Tunzi 1968; Ulfstrand 1968; Carlsson et al. 1977; Sheldon and Oswood 1977; Oswood 1979). Brönmark and Malmqvist (1984) found that quantity of organic materials in stream seston did not explain the disjunct distribution of hydropsychid caddisflies and other filter-feeding insects in lake outlet streams, and the issue of quality changes has been limited to the observation that filter-feeders are net exporters of detritus (cf. Benke and Wallace 1980), which may explain why labile constituents (microbes and small crustaceans) exported from the lake are replaced by amorphous detritus downstream from the outlet (Maciolek and Tunzi 1968; Sheldon and Oswood 1977).

The present study was undertaken to clearly differentiate quantity and quality of organic seston in relation to the distribution of hydropsychid caddisflies in a lake outlet stream. We defined food quantity as the concentration of particulate organic carbon in the water column of the stream. Food quality was more difficult to specify, but recent studies indicate that growth of lotic insects is related to availability of foods that contain high amounts of proteins (Statzner 1978; Kondratieff and Simmons 1984) and/or lipids (Hanson et al. 1983; Cargill et al. 1985). These constituents are largely related to the microbial biomass attached to or included with less labile organic and inorganic substrates which compose the seston in a particular stream segment (cf. Cummins 1974). Despite some problems, the adenosine triphosphate (ATP) assay is probably the only convenient and reliable method to quantify microbial biomass

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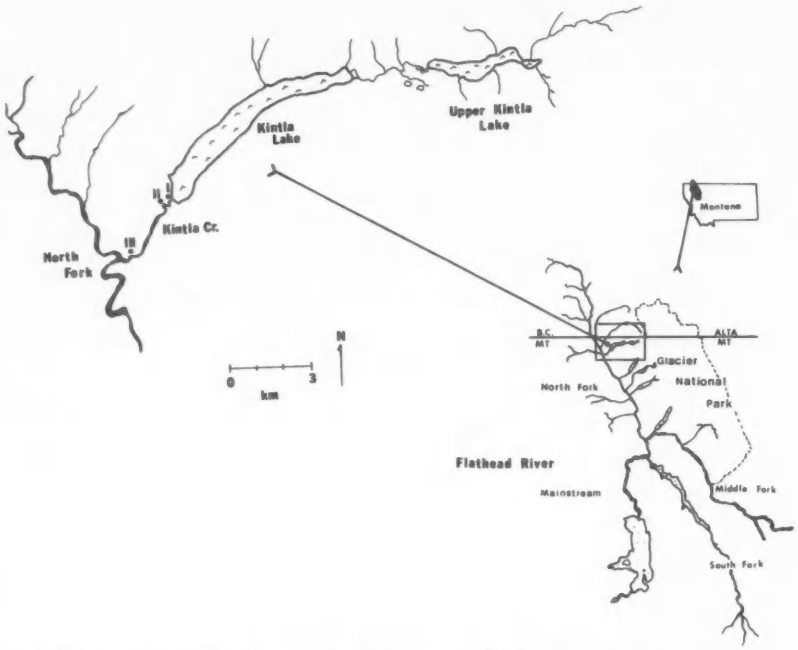

FIG. 1. Kintla Creek drainage in northwestern Montana, USA, and the study sites in the outlet stream of Kintla Lake.

in most environmental samples (Karl 1980). Moreover, ATP concentration has been found to be a reliable estimator of food quality in a variety of studies on nutritional determinants of growth in stream detritivores (cf. Ward and Cummins 1979; Wallace et al. 1982). Thus, we used particulate protein and ATP concentrations as measures of food quality. Food quantity and quality variables were measured in time series, along with important habitat variables (e.g. temperature and substratum dynamics), and correlated with measured density of the hydropsychid community in three serial stream segments downstream from a lake outlet.

Methods and Materials

Three sampling sites were established on Kintla Creek in Glacier National Park, Montana (Fig. 1). Stations I, II, and III were located respectively 10 m, 200 m, and 3.5 km downstream from the Kintla Lake outlet. Sampling frequency for all variables was somewhat limited by necessity of winter access on skis from the west side of the North Fork valley.

Discharge was measured monthly by cross-sectional integration of depth and current speed. Flows from Kintla Lake reached a minimum of 0.27 m^3/s^1 in March 1985; maximum discharge during the study period was 14.21 m^3/s^1 in June 1984. Average total alkalinity (field titration) was 60 mg $CaCO_3$/L and varied only 2 mg/L between sites. Conductivity values (YSI meter) were similar, averaging 102, 104, and 109 $\mu S/cm^2$ at sites I, II and III, respectively.

Kintla Lake was ice-covered from January to March 1985, but the outlet remained ice free for several hundred metres downstream. Ice frequently spanned the channel downstream at site III. Temperature data for the different sites were recorded using continuous recording thermographs at stations I and III. Temperature in the outlet was continually 2–4°C higher than at site III: annual degree-days accumulated at site III was only 2028, compared with 2646 in the lake outlet.

Substrate disturbance (e.g. displacement of rocks by freshets and other natural events) was rarely observed in the outlet. Substratum was typically anchored by heavy growths of the filamentous green alga, *Zygnema*, and by mats of the stalked diatom, *Gomphonema*. The algal mat was significantly reduced at site II, but large amounts of the benthic moss, *Fissidens* sp., were present. Periphyton was limited to a thin epilithic diatom film at site III, and the substratum was composed of loose gravel and cobbles. Considerable rearrangement of substratum occurred during high flows and as a result of ice scour at this site.

The presence of moss at site II may also have been indicative of CO_2-rich groundwater intrusion (cf. Hynes 1970a) below the outlet proper. Site III averaged 40% more discharge than was measured at site I; this further suggested that interstitial flows from the lake may enter the creek well downstream from the outlet, since no permanent surface tributaries enter Kintla Creek between the lake and the North Fork of the Flathead River.

Particulate organic carbon (POC) concentration in seston was measured monthly from grab samples (three per site, $N = 27$) taken in the middle of the water column and within the stream thalweg. Values were determined by a CO_2 liberation procedure (Menzel and Vaccaro 1964) on an Oceanography International 0524B total carbon system.

Protein samples (three replicate grab samples from the thalweg, $N = 81$) were collected about monthly from each of the sample locations. Samples were filtered in the field using

a hand-operated vacuum pump at 207 kPa (30 psi) maximum. The Brilliant Blue G-250 protein analysis procedure (Bradford 1976) and the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA) were used to quantify extractable particulate protein in the seston samples. We utilized this assay as modified by Beardon (1978) and Setchell (1981).

One-litre grab samples for ATP assays (three replicates per site, $N = 81$) were collected about monthly from each station. Samples were analyzed in the laboratory within 12 h after collection. We used a cold-extraction technique (Turner Instruments, Mountain View, CA) and a Lab-Line ATP photometer (Perry et al. 1979) to assay ATP concentration.

Benthic invertebrates along the Kintla outlet stream were sampled ($N = 22$) in October and December using a modified kicknet procedure (Hauer and Stanford 1981). The life histories of the hydropsychids as documented elsewhere in the Flathead River Basin suggested that maximum larval densities would normally be encountered in fall and/or midwinter (Hauer and Stanford 1982a). Benthic matter and insects collected in the net were preserved with 10% formaldehyde. Samples that were to be used for gut analysis were first inundated with bicarbonate to asphyxiate larvae and prevented regurgitation of gut contents. Hydropsychids were sorted by species and enumerated in all samples. Identifications were based on a reference collection from Hauer and Stanford (1982b).

Larval foreguts from November and December 1983 collections ($N = 72$) were examined following the technique described by Fuller and Mackay (1981) to determine the diet of hydropsychids at the three sites. Foregut contents of three hydropsychid larvae were combined for each cleared filter. Drawings, via camera lucida, of eight fields from each slide were made of gut materials, which were classified into the following food types: (1) animal matter, (2) identifiable plant matter, and (3) detritus. Foregut composition was reported as percent area of food type.

Results and Discussion

Hydropsychid Distribution

Total number of hydropsychid larvae decreased dramatically with distance downstream from the lake outlet (Fig. 2). The geometric mean ($N = 8$) for site I was 1499 larvae/m^2 whereas 200 m downstream at site II the geometric mean dropped to 287 larvae/m^2. The geometric mean for site III was 113 larvae/m^2. Statzner (1978) also noted a drastic decrease in larval densities within a similar distance downstream from a lake in West Germany.

Species composition within the Hydropsychidae also changed as distance from the outlet increased (Table 1). *Cheumatopsyche* sp. were clearly the dominant larvae in the Kintla Lake outlet; *Hydropsyche cockerelli* larvae were found only sparsely in the outlet and were the only other hydropsychid collected at that site. At site II, *H. cockerelli* comprised over half of the comparatively few larvae that were present, while *Cheumatopsyche* sp. accounted for 25% of the total. *Hydropsyche oslari* and *H. occidentalis* were also present. No *Cheumatopsyche sp.* and very few *H. cockerelli* were collected from site III; *H. oslari* and *H. occidentalis* dominated the comparatively sparce hydropsychid community at this downstream site.

Similar sequential changes in hydropsychid fauna have been observed elsewhere in the Flathead River system (Hauer et al. 1980) and in other rivers (Hildrew and Edington 1979). These researchers placed temperature and food abundance as primary controls on the distribution of individual species within the stream continuum.

However, the presence of moss at site II may have also provided excellent habitat for hydropsychids. Oswood (1979) found significantly greater numbers of hydropsychid larvae associated with moss in Owl Creek, another Montana lake outlet system. This suggested that hydropsychid population densities at site II may have been greater than expected, if moss were not present.

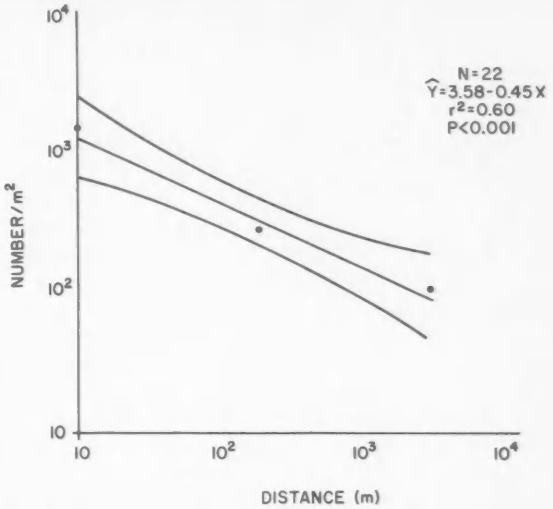

FIG. 2. Abundance of hydropsychid larvae regressed against distance downstream from the Kintla Lake outlet. Data are log-transformed geometric means of all samples collected at each of three sites in October and December 1983. Confidence limits (95%) are indicated by curved lines.

TABLE 1. Distribution of hydropsychid larvae in Kintla Creek downstream from the Kintla Lake outlet in December 1983. Data are means ± 1 SD.

Species	Distance downstream		
	10 m ($N = 4$)	200 m ($N = 4$)	3.5 km ($N = 2$)
Cheumatopsyche sp.	5522±1672	365±299	0
Hydropsyche cockerelli	32±54	800±592	10±3
Hydropsyche oslari	0	140±78	423±211
Hydropsyche occidentalis	0	126±61	151±50

Food Quality and Quantity

Particulate organic carbon values were generally the same at all sites, except during May and June 1984 when significantly greater amounts of POC were found at site III (Table 2). Only in September 1984 were POC concentrations significantly higher in the outlet and at site II than downstream at site III. In January 1985, POC values were significantly higher in the

TABLE 2. Relationship between POC content (mg C/L) of the seston and distance from the lake outlet. Boxed data (means) were not significantly different ($P < 0.01$) in Newman–Keul's multiple range tests for data grouped by sampling date (month).

Site		Mar.	Apr.	May	June	July	Aug.	Sept.	Nov.	Jan.
I	$\bar{X}$	0.081	0.160	0.167	0.164	0.114	0.245	0.308	0.067	0.132
(0.01 km)	95% CI	0.035	0.064	0.055	0.126	0.042	0.022	0.025	0.047	0.094
II	$\bar{X}$	0.056	0.186	0.173	0.214	0.150	0.270	0.285	0.246	0.143
(0.20 km)	95% CI	0.042	0.034	0.087	0.006	0.045	0.030	0.049	0.295	0.027
III	$\bar{X}$	0.017	0.193	0.248	0.222	0.141	0.248	0.258	0.104	0.061
(3.5 km)	95% CI	0.063	0.584	0.052	0.004	0.059	0.002	0.022	0.099	0.025

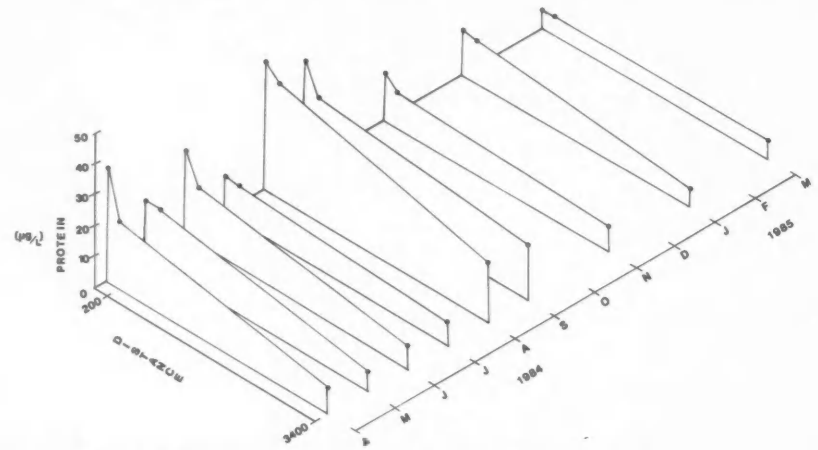

FIG. 3. Relationship between mean monthly concentration ($N = 3$ at each site) of particulate protein in seston and distance from the lake outlet.

TABLE 3. Linear regressions and coefficients of determination (r^2) for particulate protein concentrations (Y) as a function of distance downstream (X) from the Kintla Lake outlet on each sampling date. Data ($N = 3$ at each site per month) were log-transformed. Significance levels: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $P > 0.05$ (ns).

Month	Regression equation	r^2	P level
April 1984	$Y = 44.08 - 9.90X$	0.92	***
May	$Y = 26.28 - 4.98X$	0.61	*
June	$Y = 35.40 - 7.90X$	0.77	**
July	$Y = 12.50 - 1.21X$	0.19	ns
August	$Y = 52.80 - 8.52X$	0.61	*
September	$Y = 40.90 - 6.51X$	0.87	**
November	$Y = 16.80 - 2.15X$	0.94	**
January 1985	$Y = 19.10 - 3.4X$	0.66	*

outlet as compared with concentrations at site III.

Total particulate protein concentrations were consistently higher at the upstream sites (Fig. 3) on all but one sampling date (Table 3). This differed markedly with Statzner (1978), who found no significant changes in the protein concentrations downstream from a lake outlet.

During June, July, and September 1984, ATP concentrations at site I were significantly higher than at site II or III; but in January 1985, significantly less ATP was present downstream at site III (Table 4). On all other sampling dates, ATP values were similar at all sites.

Analysis of 30 larval foreguts indicated that percent animal matter remained relatively constant between sites, while amorphous detritus content increased from 46% at site I to 63% at site II and a maximum of 86% at site III. Relative amount of recognizable plant matter decreased with distance downstream; plant matter comprised 47, 28, and 12% of total gut contents at sites I, II, and III, respectively.

We concluded that while the total concentration of seston (food quantity) was about equal between sites downstream from Kintla Lake, a measurable decrease in the labile food items (food quality) was apparent at least between the two upstream sites and site III. Protein and ATP data suggested that the biotic component of the seston originating from the lake (zoo- and phytoplankton) decreased significantly within 200 m downstream from the outlet.

Ecological Ramifications

Recent work suggests that food quality and quantity (Brönmark and Malmqvist 1984; Kondratieff and Simmons 1984; Richardson 1984) and species interactions (Statzner 1978) may explain the density and distribution of the filter-feeding zoo-

TABLE 4. Relationship between ATP content (ng/L) of the seston and distance from the lake outlet. Boxed data (means) were not significantly different ($P < 0.01$) in Newman–Keul's multiple range tests for data grouped by sampling data (month).

Site		Mar.	Apr.	May	June	July	Sept.	Nov.	Jan.	Mar.
I	$\bar{X}$	0.554	3.920	1.220	4.560	2.060	2.950	2.020	2.510	1.060
(0.01 km)	95% CI	0.245	6.100	2.350	7.714	6.100	2.191	3.790	2.022	0.298
II	$\bar{X}$	0.617	1.090	3.070	1.590	0.830	1.300	2.340	2.920	1.890
(0.20 km)	95% CI	0.362	1.560	7.580	1.040	0.893	1.360	1.390	0.446	1.960
III	$\bar{X}$	0.442	1.820	1.140	0.880	1.030	1.430	2.030	0.932	1.280
(3.5 km)	95% CI	0.302	2.700	0.573	0.174	2.130	3.500	0.431	2.330	0.471

benthos of lake outlet systems. Additionally, Müller (1982) demonstrated that adult caddisflies below a lake outlet will fly upstream and oviposit on the lake surface. He suggested that the withdrawal current eventually returns the larvae to the outlet, thus maintaining high population densities. Inherent in Müller's "colonization cycle" concept is the existence in the outlet of a substantial amount of high-quality food to support higher accumulation of biomass and the attendant energy requirements for metamorphosis. Our data show that such a resource base exists for caddisflies located in the Kintla Lake outlet.

However, food quality and stream temperature may interact to determine insect distributions (Cummins 1973; Anderson and Cummins 1979; Ward and Cummins 1979; Cummins and Klug 1979; Fuller and Mackay 1980; Hanson et al. 1983). The rate of microbial production and decomposition, and hence the supply of high-quality food, may be temperature dependent. As a measure of food quality, Naiman (1984) reported different respiration rates associated with size classes of seston collected from streams of different orders. He did not, however, address the effects of the different thermal regimes from which the seston was collected. Anderson and Cummins (1979) stressed that temperature will affect aquatic insects directly by affecting animal metabolic rates and indirectly by establishing food quality and quantity. Ward and Stanford (1982) maintained that the general distribution of all aquatic insects is primarily due to the thermal regimes encountered, although food availability can limit abundance (e.g. species dependent on leaf litter will be scarce or absent in areas where leaf litter is absent). Biomass and productivity may be related to food quantity or quality interacting with temperature constraints on metabolism. The downstream reaches of river systems are generally warmer than upstream portions (Hynes 1970a), and due to greater heat capacity, larger channels manifest a more constant thermal environment (Ward 1984). Similarly, the large heat capacity of lakes contributes a more stable daily and annual thermal regime in their outflow streams (Ulfstrand 1968; Hynes 1970a). Indeed, Hynes (1970b) stated that "the observed distribution of some lake outlet aquatic insects is due primarily to the thermal regime." We concluded that the hydropsychid distribution in Kintla Creek may, therefore, be directly dependent on temperature, while population density may be determined by food quality, as suggested by Cummins (1974), Statzner (1978), and Ward and Stanford (1982).

Our data also showed that continuous input of lake-derived and largely organic seston (allochthonous matter) lends stability (cf. Fisher and Likens 1973) to outlet streams. Because large particles, including inorganic sediment, are trapped by the lake, outlet streams are not intensely scoured during spates. This lack of severe substratum disturbance allows mats of benthic algae to grow, which in turn may stabilize the stream bottom (Armitage 1976). In many ways (e.g. substratum, temperature, food quality), the outlet may be considered a predictable or constant environment in which biomass may maximize (cf. Ward and Stanford 1983). Quite likely, species interactions (e.g. predation) may also influence temporal standing crops and, perhaps, "fine tune" species richness (cf. McAuliffe 1983; Stanford and Ward 1983) in outlet streams.

In this study of the Kintla Lake outlet system, the serial replacement of *Cheumatopsyche* sp. by the three *Hydropsyche* species reflects the existence of different thermal, substratum, and food regimes within this relatively short stream segment. The distribution of these caddisflies in Kintla Creek may be determined by the species' ability to survive in a given thermal regime, while population densities may be determined more by environmental stability and the availability of high-quality food.

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Nutrient Enrichment Studies in a Coastal Plain Estuary: Changes in Phytoplankton Species Composition

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Natural phytoplankton assemblages from the Patuxent River estuary were maintained in outdoor, large-volume, continuous cultures and subjected to nutrient enrichment in a series of experiments over an 18-mo period. Nitrogen (either ammonium or nitrate) enrichment during summer and fall produced large changes in dominant species and patterns of species successions, yielding an assemblage much different from unenriched controls: strong, continued dominance by small centric diatoms at the expense of predominant flagellate species. Succession of dominant species occurred very rapidly, within 2–4 d after enrichment began. Increases in cell densities and the changes in successional patterns were caused by increased diatom growth rates rather than reduced growth rates of other dominant species. During winter and spring, N enrichment did not cause a response in the phytoplankton assemblage. Twice during winter experiments, assemblages exhibited a weak, delayed response to P; however, P enrichment did not affect individual species' growth rates or species succession at any time of year. Thus, further enrichment can affect phytoplankton growth and community structure, even in nutrient-enriched estuaries.

Les assemblages naturels de phytoplancton dans l'estuaire de la rivière Patuxent ont été gardés en culture continuelle, extérieure, à grand volume et ont servi à une série de tests d'enrichissement en éléments nutritifs sur 18 mois. L'enrichissement à l'azote (sous forme d'ammonium ou de nitrate) durant l'été et l'automne ont conduit à d'importants changements de dominance et de succession spécifiques, de telle sorte qu'il s'est recréé un assemblage très différent des assemblages témoins : une dominance marquée et prolongée de petites diatomées à symétrie radiale aux dépens des espèces flagellées prédominantes. La succession des espèces dominantes s'est produite très rapidement, entre 2 et 4 jours après le commencement des expériences. Les accroissements de densité et les modifications des successions résultent de la vitesse accrue de multiplication des diatomées plutôt que d'un ralentissement de la multiplication des autres espèces dominantes. Durant l'hiver et le printemps, l'enrichissement au N n'a suscité aucune réponse de l'assemblage phytoplanctonique. Deux fois durant les tests faits l'hiver, les assemblages ont montré une faible réponse, retardée, au P; cependant, l'enrichissement au P n'a pas eu d'effet sur les taux de multiplication d'espèces individuelles ou sur les successions d'espèces en aucun moment de l'année. Bref, tout nouvel enrichissement peut agir sur la multiplication du phytoplancton et sur la structure de la communauté, même dans des estuaires riches en éléments nutritifs.

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Phytoplankton communities undergo a continual succession of dominant species. A wide range of environmental factors can influence species dominance and succession, including light (Ryther 1956; Chan 1978, 1980), temperature (Eppley 1972; Goldman and Carpenter 1974; Goldman and Ryther 1976; Goldman and Mann 1980), and nutrient concentrations, ratios, and chemical form (Menzel et al. 1963; Schelske and Stoermer 1971; Dunstan and Tenore 1974; Goldman and Stanley 1974; Maestrini and Bonin 1981). Of these, the least predictable and most subject to alteration by man is nutrient flux and chemical form. Because phytoplankton species exhibit differing requirements for the various nutrients, changes in community structure occur as the relative nutrient concentrations and fluxes change (Tilman 1977, 1980; Kilham and Kilham 1984).

Recently, important generalizations concerning phytoplankton response to altered nutrient regimes have emerged. Several researchers (Harrison and Davis 1979; Turpin and Harrison 1979; Harrison and Turpin 1982; Kilham and Kilham 1984) have proposed that specific nutrient flux and nutrient ratios are important environmental factors influencing the dominance of various taxonomic groups (i.e. high nutrient flux promotes diatom dominance; low nutrient flux promotes flagellate dominance), while nutrient patchiness or chemical form of the nutrient may be influencing the success of one particular species over another (e.g. *Chaetoceros* spp. vs. *Skeletonema costatum*). However, we know little about how seasonality in natural ecosystems, with attendant variation in light, temperature, nutrient flux, and dominant phytoplankton species, affects these general nutrient responses.

TABLE 1. Environmental and test parameters for each experiment.

Experiment No.	Season	Duration (d)	Enrichments ($\mu mol \cdot L^{-1}$)		Dilution ($\% \cdot d^{-1} \pm SD$)	Ambient T (°C)	Salinity range (‰)
8–83	Summer	15	NH_4^+	100	50	26–28	9.5–10.0
			PO_4^{3-}	20			
			$NH_4^+ + PO_4^{3-}$	100 + 20			
10–83	Fall	19	NH_4^+	50	47±5	9–12	9.8–10.5
			NO_3^-	50			
			PO_4^{3-}	10			
			$NH_4^+ + NO_3^-$	25 + 25			
2–84	Winter	19	NH_4^+	50	45±3	6–9	6.0–8.5
			NO_3^-	50			
			PO_4^{3-}	10			
3–84	Early spring	22	NH_4^+	50	51±2	7–12	3.5–6.0
			NO_3^-	50			
			PO_4^{3-}	10			
4–84	Late spring	22	NH_4^+	50	50±2	15–25	3.2–4.5
			NO_3^-	50			
			PO_4^{3-}	10			
6–84	Summer	19	NH_4^+	50	51±2	24–29	4.2–5.5
			NO_3^-	50			
			PO_4^{3-}	10			
7–84	Late summer	15	NH_4^+	50	51±1	26–23	7.0–8.5
			NO_3^-	50			
			PO_4^{3-}	10			
8–84	Early fall	16	NH_4^+	50	51±2	25–17	7.0–8.0
			NO_3^-	50			
			PO_4^{3-}	10			
9–84	Fall	30	NH_4^+	50	50±1	20–11	6.0–8.2
			NO_3^-	50			
			PO_4^{3-}	10			

Over a 2-yr period, we conducted a series of large-volume, continuous culture experiments designed to investigate the response of phytoplankton to nutrient addition in an estuary subjected to considerable nutrient enrichment. Our overall objectives were to (1) determine whether natural phytoplankton assemblages, over the annual cycle, were N or P limited (and therefore would respond to additions of these nutrients) and (2) study the changes in species composition within the phytoplankton community in response to nutrient additions, in the various seasons. D'Elia et al. (1986) addressed the first objective; here we present the second.

Materials and Methods

Natural phytoplankton from the Patuxent River (a subestuary of the Chesapeake Bay) were cultured outdoors in 500-L, temperature-controlled, cylindrical fiberglass tanks. The tanks operate as continuous, flow-through phytoplankton cultures using mesohaline river water as the diluent. This experimental system has been used extensively and is discussed elsewhere (Sanders et al. 1981; Sanders and Vermersch 1982; Sanders and Cibik 1985; D'Elia et al. 1986). The tanks were initially filled with estuarine water containing the natural phytoplankton assemblage after passage through 35-μm nylon mesh to remove large herbivores. This initial screening did not remove a significant fraction of the phytoplankton. After filling, each tank was sampled and the phytoplankton species composition and abundance compared between tanks as a measure of tank-to-tank variability (Sanders et al. 1981). Filtered (1-μm) water was then continuously supplied to each tank at a nominal dilution rate of 50% per day (Sanders and Cibik 1985; D'Elia et al. 1986) and continuous nutrient infusions were begun. Concentrated solutions of P and N were added to triplicate sets of tanks to maintain elevated nutrient concentrations in the dilution water (10 μM PO_4^{3-} or 50 μM NO_3^- or 50 μM NH_4^+; see Table 1 for exceptions). Actual nutrient concentrations within the tanks varied depending upon background nutrient concentrations and the extent of algal incorporation of added nutrients.

Once initiated, the cultures were maintained as above for 2–4 wk. Daily water samples were taken from each tank for

TABLE 2. Phytoplankton response to nutrient enrichment. Positive responses are proportional to increase in cell densities and biomass, with a weak response equivalent to variable increases less than 2 times control, moderate response signifying a constant increase of 2 times control, and a strong response signifying a constant increase of >2 times control.

Experiment No.	Season	Nutrient input	Generalized response
8–83	Summer	PO_4^{3-}	No response
		NH_4^+	Strong, immediate response
		$NH_4^+ + PO_4^{3-}$	Strong, immediate response
10–83	Fall	PO_4^{3-}	Weak response after 10 d
		NO_3^-	Weak response after 10 d
		NH_4^+	Moderate response after 2 d
		$NH_4^+ + NO_3^-$	Moderate response after 2 d
2–84	Winter	PO_4^{3-}	Weak response after 10 d
		NO_3^-	No response
		NH_4^+	No response
3–84	Spring	PO_4^{3-}	No response
		NO_3^-	No response
		NH_4^+	No response
4–84	Spring	PO_4^{3-}	No response
		NO_3^-	No response
		NH_4^+	Weak response after 10 d
6–84	Summer	PO_4^{3-}	No response
		NO_3^-	Strong response within 4 d
		NH_4^+	Strong response within 2 d
7–84	Summer	PO_4^{3-}	No response
		NO_3^-	Strong, immediate response
		NH_4^+	Strong, immediate response
8–84	Fall	PO_4^{3-}	No response
		NO_3^-	Strong, immediate response
		NH_4^+	Strong, immediate response
9–84	Fall	PO_4^{3-}	No response
		NO_3^-	No response
		NH_4^+	No response

phytoplankton identification and enumeration using a sedimentation technique (Utermöhl 1958); three per week were examined, and the remainder were archived for later examination if required. All daily sampling was performed at the same time each day. Phytoplankton growth rates (Eppley and Strickland 1968) were calculated from these data, yielding division rates averaged over a period of 1+ d.

A high degree of replicability within treatments is required in these experiments to separate natural from experimental variability and is routinely achieved. Replicate tanks typically vary less than 20% throughout the course of an experiment, somewhat less than the potential error of techniques employed for phytoplankton identification and enumeration (Vernick 1978). Further details may be found in D'Elia et al. (1986).

Nine experiments were performed between June 1983 and November 1984 (see Table 1).

Results

Response to Nutrient Enrichment

The general response of natural phytoplankton assemblages to nutrient enrichment (as measured by an increase in biomass) was considered previously (D'Elia et al. 1986) and is only summarized here (Table 2). During the summer and early fall there were rapid, large responses to N, but not P, enrichment; algal densities in N-enriched cultures doubled relative to control or P-enriched cultures within 24 h and often peaked at densities 7–15 times higher than controls within 3–7 d. During late fall, winter, and spring, responses to N or P enrichment, if any, were weak and slow.

Species Composition

Changes in dominant species and groups varied with time in

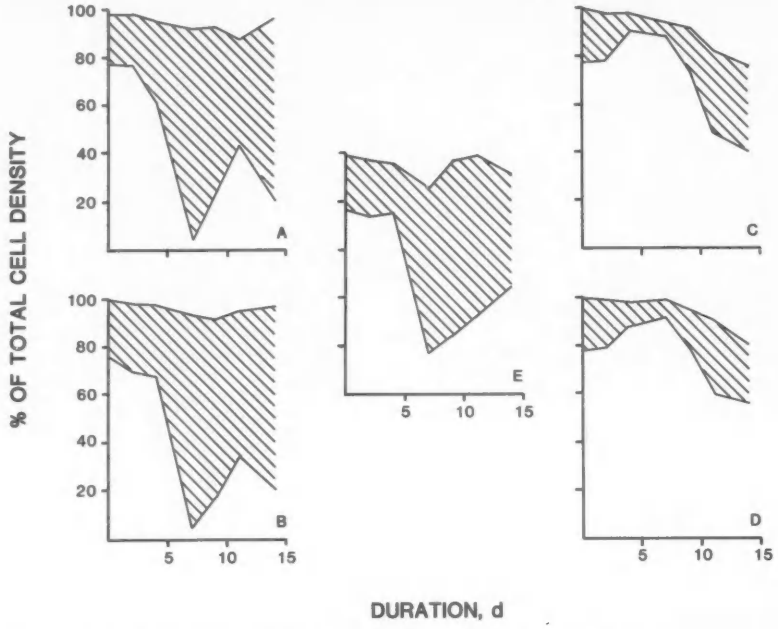

FIG. 1. Relative proportions of two important algal groups, centric diatoms (□) and naked flagellates (▨), through time in tank and natural river assemblages exposed to NH_4^+, NO_3^-, PO_4^{3-}, or no nutrient enrichment. Experiment 8-84 (early fall). (A) Control; (B) PO_4^{3-}; (C) NO_3^-; (D) NH_4^+; (E) river.

TABLE 3. Dominant species during summer and early fall experiments (strong response to N enrichment). Only species which constituted greater than 10% of total cell density for at least 75% of the experiment duration are listed. Dominants are listed in order of abundance.

Experiment No.	No enrichment	P enrichment	N (NH_4^+) enrichment	N (NO_3^-) enrichment
8–83	*Chaetoceros* spp. *Cylindrotheca closterium* *Thalassiosira pseudonana* Small flagellates	Small flagellates *Thalassiosira pseudonana* *Cylindrotheca closterium*	*Thalassiosira pseudonana* *Cylindrotheca closterium* Small centrics *Cyclotella* sp.	
6–84	Small flagellates Small centrics *Skeletonema costatum*	Small flagellates Small centrics *Skeletonema costatum*	*T. pseudonana* Small centrics *Skeletonema costatum*	*Skeletonema costatum* Small centrics Small flagellates
7–84	Small flagellates *C. closterium* *S. costatum* Small centrics	Small flagellates *C. closterium* *S. costatum* Small centrics	*S. costatum* Small flagellates *C. closterium* Small centrics	*S. costatum* Small centrics *Cylindrotheca closterium* Small flagellates
8–84	Small flagellates *Chroomonas* spp.	Small flagellates *Chroomonas* spp.	*Thalassiosira nordenskioldii* Small flagellates	*Thalassiosira nordenskioldii* Small flagellates

all experiments. There was considerable seasonal variation, however, in responses to nutrient enrichment; these responses are discussed separately by season below.

Summer–fall experiments

Changes in species composition during summer and early fall experiments were quite dynamic. Species succession in control cultures and cultures enriched with P was similar; assemblages were dominated by small flagellate species and centric diatoms such as *Thalassiosira pseudonana*, *S. costatum*, and *Chaetoceros* spp. (Table 3). Similar successional changes also occurred within the Patuxent River. Flagellates were in general numerically more abundant than centric diatoms both in the control and P-enriched cultures and within the Patuxent River (Fig. 1).

Nitrogen enrichment, either as NO_3^- or NH_4^+, promoted strong dominance by centric diatoms such as *S. costatum*, *Cyclotella* spp., *T. pseudonana*, and *Thalassiosira nordenskioldii*, yielding an assemblage much different from those in control and P-enriched cultures and the Patuxent River (Fig. 1; Table 3). Succession of dominant species occurred very rapidly in N-enriched cultures, with one species strongly dominant in the assemblage after 2–4 d, as was the case with *S. costatum* in experiment 7–84 or *T. nordenskioldii* in experi-

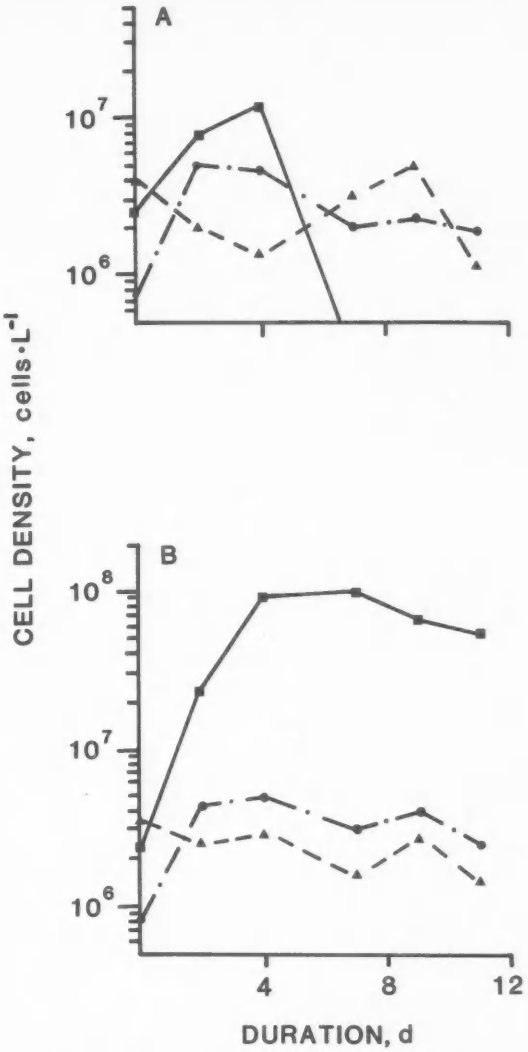

FIG. 2. Succession of dominant species, in terms of cell density, through time in assemblages exposed to NH_4^+, NO_3^-, PO_4^{3-}, or no nutrient enrichment. Experiment 8-84 (early fall). (A) Control or PO_4^{3-}; (B) NO_3^- or NH_4^+. ■, *Thalassiosira nordenskioldii*; ▲, *Chroomonas* sp.; ●, small flagellates.

ment 8−84 (Fig. 2). On some occasions, these initial dominants would remain the predominant species for the remainder of the experiment; however, replacement by a similar species occurred in some experiments, such as the replacement of *S. costatum* by another small centric diatom in experiment 7−84.

The chemical form of added N appeared to affect species succession in only one experiment (6−84, Table 3). In this experiment, *S. costatum* dominated nitrate-enriched cultures, as well as control and P-enriched cultures after the first 5 d. In the ammonium-enriched cultures, however, *T. pseudonana* was the dominant species, with a small, unidentified centric and *S. costatum* also important. No other differences were

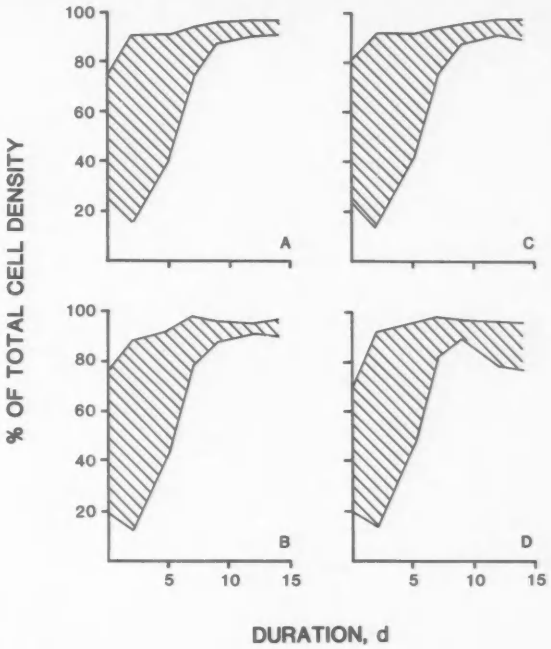

FIG. 3. Relative proportions of two important algal groups, centric diatoms (□) and naked flagellates (▨), through time in assemblages exposed to NH_4^+, NO_3^-, PO_4^{3-}, or no nutrient enrichment. Experiment 3−84 (early spring). (A) Control; (B) PO_4^{3-}; (C) NO_3^-; (D) NH_4^+.

noted between nitrate- and ammonium-enriched cultures.

The increase in total cell density and the changes in successional patterns in N-enriched cultures in summer−fall experiments were caused by increased diatom growth rates, rather than reduced growth rates of other species. For example, the initial growth rate of *S. costatum* and the overall growth rate of an unidentified centric in experiment 7−84 were increased 59 and 110% in N-enriched tanks relative to their growth in controls; the growth rate of the entire assemblage was increased 74%. In experiment 8−84, the initial growth rate (first 4 d) of *T. nordenskioldii* was increased 65% by N enrichment. The growth rate of the entire assemblage was increased by 100%. Diatom growth rates in P-enriched cultures in all experiments were the same as controls. Growth rates of dominant flagellate species also were unaffected by nutrient enrichment regardless of treatment or season (Table 4).

Winter−spring experiments

Species succession in control and nutrient-enriched assemblages followed a similar pattern during late fall, winter, and spring experiments. During this period, assemblages were dominated primarily by small centric diatoms, such as *S. costatum*, *Chaetoceros* spp., and *T. pseudonana*, small flagellates such as *Chroomonas* spp., and the dinoflagellate, *Katodinium rotundatum*, during late winter − early spring (Table 5); phytoplankton within the Patuxent River were dominated by the same species. Nutrient enrichment did not alter this successional pattern or growth rates of dominant species. The dominance of two groups, centric diatoms and, to a lesser extent,

TABLE 4. Average growth rates (μ, divisions·d^{-1}) of dominant species and phytoplankton assemblages under varying nutrient enrichments in selected experiments. Ambient temperatures and predicted maximum growth rates (Eppley 1972; Goldman and Carpenter 1974) are also noted.

Experiment No.	Temperature range (°C)	Predicted μ_{max}	Species	μ Control	μ PO_4^{3-}	μ NO_3^-	μ NH_4^+
3–84	7–12	1.6	*Thalassiosira decipiens*	1.2	1.2	1.2	1.3
			Unidentified microflagellates	0.7	0.7	0.7	0.8
			Katodinium rotundatum	0.7	0.7	0.6	0.7
			Whole assemblage	1.0	1.0	1.0	1.0
7–84	23–26	4.2	*Skeletonema costatum*	1.5	1.5	2.3	2.4
			<30 μm centric diatoms	0.5	0.5	1.0	1.1
			Unidentified microflagellates	0.3	0.2	0.1	0.2
			Whole assemblage	0.6	0.6	0.9	0.9
8–84	17–25	3.2	*Thalassiosira nordenskioldii* (first 4 d)	1.1	1.2	1.8	1.8
			Chroomonas sp.	0.5	0.5	0.5	0.5
			Unidentified microflagellates	0.7	0.6	0.8	0.7
			Whole assemblage (first 4 d)	0.5	0.5	1.0	1.0
			Whole assemblage (overall)	0.4	0.4	0.8	0.8

TABLE 5. Dominant species during late fall, winter, and spring experiments (generally no or weak response to nutrient enrichment). Only species which constituted greater than 10% of the total cell density for at least 75% of the experiment duration are listed. Dominants are listed in order of abundance.

Experiment No.	No enrichment	P enrichment	N enrichment
10–83	*Skeletonema costatum*	*Skeletonema costatum*	*Skeletonema costatum*
	Small flagellates	Small flagellates	Small flagellates
	Chroomonas spp.	*Chroomonas* spp.	*Chroomonas* spp.
2–84	*S. costatum*	*S. costatum*	*S. costatum*
	Small centrics	Small centrics	Small centrics
	Chaetoceros sp.	*Chaetoceros* sp.	*Chaetoceros* sp.
	Small flagellates	Small flagellates	Small flagellates
3–84	*Thalassiosira decipiens*	*Thalassiosira decipiens*	*Thalassiosira decipiens*
	Small flagellates	Small flagellates	Small flagellates
	Katodinium rotundatum	*Katodinium rotundatum*	*Katodinium rotundatum*
4–84	*Dictyosphaerium* sp.	*Dictyosphaerium* sp.	*Dictyosphaerium* sp.
	Thalassiosira pseudonana	*Thalassiosira pseudonana*	*Thalassiosira pseudonana*
			Small centrics
9–84	*Chaetoceros debile*	*Chaetoceros debile*	*Chaetoceros debile*
	Small flagellates	Small flagellates	Small flagellates
	S. costatum	*Chroomonas* sp.	*S. costatum*
	Chroomonas sp.	*S. costatum*	*Chroomonas* sp.

naked flagellates, was quite apparent, as was the similarity between treatments (Fig. 3).

Discussion

The seasonal shift in algal response to nutrient enrichment in these experiments was quite striking, although predictable by indirect means. Summer is the period of lowest N concentrations and N:P ratios in the Patuxent River estuary (Flemer et al. 1970; D'Elia et al. 1986); thus, the potential for N limitation is at a maximum. Winter nutrient concentrations are generally high enough and levels of insolation low enough to expect light, not nutrient, limitation in this turbid estuary. Similar seasonal variation in phytoplankton response to nutrient additions has been noted for the Delaware estuary (Pennock 1985) and the York River in the lower Chesapeake Bay (K. L. Webb, Virginia Institute of Marine Sciences, Gloucester Point, VA 23062, unpubl. data).

Although the response of the algal assemblages to N enrichment in the summer–fall experiments differed somewhat in magnitude from month to month and year to year, the general pattern was the same: one or two dominant centric diatoms rapidly increased their growth rate relative to other dominant species and to growth rates in control and P-enriched assemblages, thereby greatly increasing their dominance and the total cell density of the assemblage. Although growth rates

of other dominant species remained unchanged, they rapidly became less important within the assemblage as the dominant diatoms increased in density.

Diatoms may inherently grow faster than flagellates and other unicellular algae when nutrients and other environmental conditions are favorable (Pearsall 1932; Chan 1978, 1980; Parsons et al. 1978; Thomas et al. 1978). The large increases in growth rate of centric diatoms in these experiments are consistent with this concept. Diatom growth rates were as much as doubled by the addition of N during summer and fall (Table 4); similar increases in growth rate have been measured elsewhere (Takahashi et al. 1982). Some algal species have the ability to adapt rapidly to changing nutrient regimes, altering their growth rate and nutrient uptake to meet these altered conditions (Droop 1974). This shift in physiological state has been demonstrated for *S. costatum* (Harrison et al. 1976; Conway et al. 1976); the results presented here suggest that such a shift may be more common to diatoms than to flagellates.

The large-volume cultures utilized in these experiments do not function as chemostats because algal growth can vary considerably on a daily basis in response to environmental variables. Growth rates reported here are averaged over a period of 1–10 d and therefore approximate a maximum sustainable rate under the prevailing nutrient conditions. Growth rates measured during these experiments generally were in the range of 0.5–1.0 divisions·d^{-1}; with N enrichment, rates of diatom growth often exceeded 1.0 divisions·d^{-1}, approaching 2.0 divisions·d^{-1} in species of *Thalassiosira* and 2.5 divisions·d^{-1} in *S. costatum* (Table 4). Without exception, these rates were considerably less than theoretical or measured maximum growth rates (Eppley 1972; Goldman and Carpenter 1974), undoubtedly reflecting the difference between laboratory culture conditions and conditions in natural ecosystems, where physical processes and light intensity and quality are variable and usually less than ideal.

The results presented here are generally in accord with the current theory that increased nutrient (N) flux supports centric diatom dominance, but only during one period of the year, summer–early fall; increases in nutrient flux had no impact on the species composition during other seasons. However, phytoplankton did not appear to respond differently to nitrate or ammonium under N-limited conditions. Increases in growth rates of dominant centric diatoms were identical under either N source (Table 4); with the exception of one experiment, dominant species and successional patterns were identical as well. Earlier studies have indicated that some diatom species may assimilate ammonium more rapidly than nitrate (Lui and Roels 1972), implying a faster response to nutrient addition, a faster growth rate, or both. Such a response was not evident in our results.

Although the objective of these experiments was not to replicate successional changes and growth rates within a natural phytoplankton community, changes within control and P-enriched cultures during each experiment and N-enriched cultures during winter and spring largely mirrored changes occurring in the nearby Patuxent River (e.g. Fig. 1). The close similarity between the experimental cultures and the Patuxent River demonstrates the suitability of the experimental design and underscores the relevance of the data presented. Although our design excludes large grazers, these results and the similarity with the Patuxent River suggest that such grazing pressure may not be an important factor regulating phytoplankton standing stocks or species succession. Recently, investigators have proposed that small (<2 μm) autotrophic species are responsible for a significant fraction of primary production in coastal marine systems. Although this fraction did not attain significant densities in our experiments, further research is necessary to closely study the microzooplankton/picoplankton trophic link.

Management Implications

The general results of this study — seasonal N limitation and increased growth of dominant centric diatoms — are of significance to the management of nutrient-stressed estuaries. These studies have shown that nutrient enrichment can affect phytoplankton growth and community structure, even in nutrient-stressed estuaries. Within the Patuxent River estuary, N is limiting (and thus capable of controlling phytoplankton dynamics) only for several months during summer and fall. During other periods of the year, phytoplankton appear to be N and P sufficient; presumably, factors such as light intensity, trace compounds such as metals and vitamins, herbivore interactions, or perhaps even temperature control community dynamics. The algal species that responded to N enrichment during summer–fall experiments were those generally classified as "beneficial" species. However, indications are that a majority of the carbon produced by phytoplankton in the highly productive Chesapeake Bay is not being consumed by herbivores; thus, higher rates of algal growth, even of "beneficial" species, are not necessarily a benefit to the estuary as a whole (D'Elia et al. 1986).

In light of increased scientific and public awareness of the potential impact of nutrient enrichment, management agencies are employing coupled numerical water quality and hydrodynamic models to predict responses to nutrient addition or to relaxation of effluent standards. Such models attempt to predict algal productivities and biomass achievable within a given system and require knowledge of phytoplankton growth rates, but there are few data available for the growth rates of estuarine phytoplankton species; available data have been derived principally from laboratory culture work and may differ considerably from actual growth rates (Table 4). This study provides data on phytoplankton growth rates achieved by dominant and subdominant species in outdoor cultures replete with nutrients and exposed to ambient light and temperature conditions, and may represent more realistic values than previous studies have provided.

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Cadmium in Sea Scallop (*Placopecten magellanicus*) Tissues from Clean and Contaminated Areas

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Uthe, J. F., and C. L. Chou. 1987. Cadmium in sea scallop (*Placopecten magellanicus*) tissues from clean and contaminated areas. Can. J. Fish. Aquat. Sci. 44: 91–98.

Over 90% of the total cadmium in the soft tissues of sea scallops (*Placopecten magellanicus*) was in the digestive gland with less than 1% in the adductor muscle. The amount of cadmium in the digestive gland was significantly related to shell height. Shell height was superior to age as an independent (predictor) variable due to difficulties in ageing scallops. Based on these relationships, scallops of approximately 100 mm shell height were selected to study interregional differences. Neither cadmium concentration nor burden could be used to identify contaminated areas. The ratio of digestive gland cadmium to that in the adductor muscle was lowest for scallops from Chaleur Bay, which had received substantial anthropogenic cadmium input, and for scallops that had been starved for approximately 14 mo. We suggest that the high tissue cadmium levels in scallops from Georges Bank and Browns Bank are not due to contamination from anthropogenic or natural sources but rather reflect feeding and the nutritional inadequacy of the diets. Conversely, the high levels of cadmium input to Chaleur Bay were not reflected in high tissue concentrations or burdens in scallops.

Plus de 90 % du cadmium des tissues mous des petoncles géants (*Placopecten magellanicus*) se trouvait dans la glande digestive avec moins de 1 % dans le muscle adducteur. La quantité de cadmium dans la glande digestive était liée de manière significative à l'épaisseur du coquillage. L'épaisseur du coquillage était une meilleure variable indépendante que l'âge qui est difficile à déterminer chez le pétoncle. À partir de ces relations, on a choisi des coquillages de 100 mm d'épaisseur pour étudier les différences inter-régionales. Ni la concentration ni la charge en cadmium n'a pu servir à indentifier les zones contaminées. Le rapport de la concentration de cadmium de la glande digestive à celle du muscle adducteur était plus faible que celui des pétoncles de la baie des Chaleurs qui avaient absorbé une quantité substantielle de cadmium anthropogène et que celui des pétoncles qui avaient été privés de nourriture pendant environ 14 mo. Les fortes teneurs en cadmium des pétoncles du banc de Georges, et en particulier du banc de Browns, ne tiendraient pas à une contamination d'origine anthropogène ou naturelle, mais plutôt aux habitudes alimentaires et à l'équilibre de leur alimentation. Par ailleurs, les décharges elevées de cadmium a la Baie des Chaleurs ne semblent pas être associées ni aux concentrations ni aux charges observées dans les tissues des petoncles.

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Over the past decade or so the use of bivalves such as mussels as sentinel indicators of coastal contamination by trace metals and other anthropogenic chemicals has grown in popularity (U.S. National Academy of Sciences 1980; Phillips 1980). Concern over the presence of trace metals in marine biota from a human health point of view has also increased, as various studies have reported rather high concentrations in some edible shellfish (e.g. Topping 1973; Bryan 1973). Cadmium is the most serious metallic contaminant with which man has to deal in the latter part of the twentieth century (Goldberg 1984).

Sandi (1979) reported that the cadmium intake from foods in Canada may slightly exceed the FAO/WHO provisional tolerable weekly intake of 0.4–0.5 mg. Freeman and Uthe (1974) reported that the edible portion of American lobster (*Homarus americanus*) captured in uncontaminated areas of the Maritime Provinces contained 0.39–1.36 mg cadmium when normalized to a 454-g (1 lb) lobster. Cadmium concentrations in lobsters captured in the area of Belledune Harbour in Chaleur Bay, New Brunswick, were high enough to warrant closing of the harbour to commercial lobster fishing in 1980 (Uthe et al. 1982). Ray et al. (1980) determined cadmium levels in other marine biota from the Belledune area. Cadmium concentrations in sea scallop (*Placopecten magellanicus*) ranged 0.5–8.4 mg/kg dry wt for muscle, 3.2–18 mg/kg dry wt for mantle, and 87.6–493 mg/kg dry wt for the remaining tissue. On a wet weight basis, concentrations in the adductor muscle averaged 0.8 mg/kg with a range of 0.1–1.6 mg/kg. These values were compared with those of Zook et al. (1976) who reported that adductor muscle of sea scallops collected off the American east coast contained an average of 0.1 mg cadmium/kg wet wt with a range of 0.04–0.20 mg/kg. Ray et al. (1980) also demonstrated higher cadmium concentrations in scallops caught "downstream" of Belledune Harbour compared with other areas of Chaleur Bay, although the number of scallops sampled in the study was small.

In a later study, Ray et al. (1984) showed that cadmium burdens in scallops from Browns Bank and Georges Bank were

TABLE 1. Mean cadmium levels (± SD) in soft parts of sea scallop (100 ± 1.3 mm shell height; $N = 6$). The relative standard deviation is given in parentheses.

Tissue	Tissue weight (g)	Cadmium concentration (mg/kg wet wt)	Cadmium tissue burden (μg)	Percent of total cadmium in soft tissues
Whole adductor muscle	16.52 ± 1.44 (8.7)	0.117 ± 0.030 (26)	1.93 ± 0.54 (28)	0.56 ± 0.12 (22)
"Quick" muscle	15.29 ± 1.27 (8.3)	0.116 ± 0.030 (26)	1.79 ± 0.52 (29)	0.52 ± 0.12 (22)
"Catch" muscle	1.23 ± 0.28 (23)	0.115 ± 0.027 (24)	0.14 ± 0.04 (31)	0.04 ± 0.01 (35)
Mantle	9.91 ± 1.00 (10)	0.408 ± 0.165 (40)	3.97 ± 1.32 (33)	1.18 ± 0.45 (38)
Gonad	8.15 ± 2.28 (28)	0.190 ± 0.099 (52)	1.41 ± 0.52 (37)	0.42 ± 0.16 (39)
Gill	5.14 ± 0.37 (7.2)	0.830 ± 0.265 (32)	4.28 ± 1.47 (34)	1.28 ± 0.48 (38)
Kidney	0.27 ± 0.06 (22)	62.61 ± 25.33 (40)	16.9 ± 8.1 (48)	5.43 ± 1.80 (33)
Digestive gland	3.33 ± 0.40 (12)	94.68 ± 8.42 (8.9)	313.8 ± 35.3 (11)	91.58 ± 2.54 (2.8)
Foot	0.14 ± 0.04 (29)	1.32 ± 1.23 (93)	0.24 ± 0.24 (99)	0.07 ± 0.06 (87)
Resilium	0.14 ± 0.03 (24)	Not detected	—	—
Total soft tissues	—	—	342.51 ± 36.56 (10.7)	—

generally higher than in Chaleur Bay scallops and reported that mean cadmium concentrations in scallop adductor muscle from Browns Bank and two sites on Georges Bank were 18.9 (5.4–36.3), 11.6 (3.2–20.2), and 5.0 (2.9–10.5) mg/kg dry wt, respectively. The authors suggested that a natural source of cadmium was responsible for the high cadmium concentrations found in these scallops. Such levels could pose a threat to the commercial scallop fisheries if health agencies deemed the product unfit for sale. Ray et al. (1984) pointed out that because of the time required to transport scallops from the banks to the laboratory, the intact animals were frozen after capture and dissected after reaching the laboratory and that there was a possibility that necrochemical changes could have led to cadmium contamination of the adductor muscle from the viscera.

To throw further light on the occurrence of cadmium in scallops, we have examined scallops from Georges Bank and Browns Bank along with two areas in Chaleur Bay (Belledune Harbour and Petit Rocher) that have received substantial input of cadmium in recent years (Uthe and Zitko 1980) and Passamaquoddy Bay, which Ray et al. (1984) considered relatively clean.

Materials and Methods

Scallops were collected by commercial scallop draggers at the locations given in Table 3 and transported live to the laboratory aquarium where they were held in flowing seawater until dissection.

Scallops were dissected, generally within 2 wk of capture, unless otherwise noted; we ensured that each intact organ was removed rapidly without cross contamination. Dissection was carried out by carefully removing the animal from its shell and suspending it above the working surface with two small clamps on the mantle so that the digestive gland was below the adductor muscle which was removed first. A small iris scissors (10 cm long) was a great help in obtaining clean dissections. We recommend that individuals attempting to dissect scallops in this manner practice a number of times (without analyzing the tissues) until the procedure can be carried out quickly and cleanly. Cadmium measurements on our own initial dissections showed that the adductor muscle could easily be contaminated, presumably through both unclean dissections and autolysis of the digestive gland.

Either whole tissues or subsamples were digested in concentrated nitric acid in 50-mL graduated Folin-Wu tubes using micro-Kjeldahl heating racks (Lab Con) for gentle heating. In the case of the muscle tissue a nitric – sulfuric acid mixture (5:3, v/v) was used. Digestion time was 1.5–2 h. After cooling, the volume of the digest was made up to 25 mL with glass-distilled water. Measurement of cadmium was carried out with atomic absorption spectrophotometry using Perkin-Elmer model 403 spectrophotometers fitted with deuterium arc background correctors. The method of standard additions was employed. A graphite tube furnace was used for those digests in which the cadmium concentration was below that required for satisfactory flame analysis. Our methods have been proved satisfactory through interlaboratory comparative studies sponsored by the International Council for the Exploration of the Sea (Holden and Topping 1981). Statistical analyses were conducted using the Standard and Statistical Pacs on a Hewlett-Packard model 97 calculator. All tests of significance were at the 5% level. Cadmium concentrations are reported in milligrams per kilogram wet weight and cadmium burdens in micrograms. Cadmium burden is defined as the amount of cadmium present in 1.0 g of tissue multiplied by the weight (grams) of the whole tissue Standard deviations are given in the tables.

Results and Discussion

Distribution of Cadmium in Scallop Soft Tissues

Cadmium levels were determined in all soft tissues from a suite of scallops from Georges Bank (Table 1). Scallops of approximately 100 mm shell height (Bourne 1964) were used. Most of the cadmium present in the soft tissues was in the digestive gland (midgut gland, hepatopancreas) which contained an average of 91.58% of the cadmium burden. The next largest amount was present in the kidney which contained an average of only 5.43% of the soft tissue burden. Average mantle and the gill burdens were 1.18 and 1.28%, respectively. The adductor muscle and the gonad, the edible tissues, contained 0.56 and 0.42%, respectively. No cadmium was detected in the resilium (hinge).

The average cadmium concentration in the adductor muscle, the usual edible portion of the scallop in the North American market, was 0.117 ± 0.030 mg/kg wet wt. The adductor muscle contains two types of muscle (Bourne 1964), a larger "quick muscle" and a smaller "catch muscle." The cadmium concentrations in these two muscles were not significantly dif-

ferent (Table 1). The adductor muscle is the largest of the soft tissues of the scallop but because of its very low cadmium concentration contained on average only 0.56% of the cadmium in the soft tissues.

Cadmium concentrations averaged 94.68 mg/kg wet wt in the digestive gland and 62.61 mg/kg in the kidney whereas concentrations in all other tissues were less than 1.0 mg/kg. This suggests that digestive gland and kidney are the ultimate storage organs for cadmium and shows that of the two, the digestive gland is the major storage site. A similar situation exists in American lobster, in which the highest cadmium concentrations are present in the digestive gland with a lower concentration in the kidney (green gland) (Ray et al. 1981). The relative sizes of the digestive gland and the kidney in both lobster and scallop result in the digestive gland in both species containing well over 90% of the soft tissue cadmium burden of the animals.

The digestive gland cadmium concentration is approximately 1000 times that of the adductor muscle. Because of the proximity of these two organs there is a real possibility of cadmium from the digestive gland contaminating the adductor muscle during dissection. Digestive glands from lobster and, presumably, scallops are quite autolytic, releasing free cadmium from its protein matrix after the death of the animal unless special precautions, such as the use of a protease inhibitor, are taken (Guy et al. 1985). Studies of frozen whole scallops (Uthe and Chou 1985) showed that portions of the adductor muscle in contact with the digestive gland contained elevated cadmium concentrations, while adductor muscle removed from living scallops had a low cadmium concentration evenly distributed throughout. We recommend that other investigators consider the possibilities of chemical changes in dead tissues and intertissue contamination and treat their samples accordingly.

Effects of Size and Other Variables on Soft Tissue Cadmium Levels

Cadmium concentrations were determined in digestive gland and adductor muscle from a range of differently sized scallops from Georges Bank (50–128 mm shell height). Since the cadmium concentrations in the two muscle types comprising the adductor muscle were essentially the same, only a portion of the "quick muscle" was analyzed from the larger animals. The average cadmium concentration in the digestive gland was 119.57 ± 37.31 mg/kg wt (N = 60, range 60.82–205.8, no outliers ($\bar{X} \pm 2.35 \times$ SD), while the average cadmium concentration in the adductor muscle was 0.092 ± 0.031 mg/kg wet wt (N = 57, range 0.043–0.192, three outliers eliminated as greater than the range set by $\bar{X} \pm 2.35 \times$ SD). The fact that there were no outliers identified in the digestive gland data and three abnormally high outliers in the adductor muscle results suggests either that a subpopulation of scallops exists in which the cadmium concentration in the muscle is abnormally high or, more unlikely, that the dissection of these three scallops led to some slight contamination of the adductor muscle.

A similar study was carried out on scallops from Passamaquoddy Bay, New Brunswick (54–114 mm shell height), an area reported by Ray et al. (1984) to yield scallops with relatively low cadmium levels. The average cadmium concentration in the digestive gland was 73.85 ± 19.54 mg/kg wet wt ($\bar{N}$ = 36, range 45.76–139.1, one high outlier), while the average cadmium concentration in the adductor muscle was 0.118 ± 0.048 mg/kg wt (N = 35, range 0.055–0.186, two high outliers).

The relationships between adductor muscle or digestive gland cadmium concentration or burden and size, age, and each other were investigated (Table 2). There was no significant relationship between adductor muscle cadmium concentration and animal size (shell height) for either Georges Bank or Passamaquoddy Bay scallops. A highly significant, positive log–log (power) relationship between cadmium concentration in digestive gland and shell height was found for both sample sites, showing that cadmium concentration in the digestive gland increased in an accelerating manner as the animal grew (Boyden 1977).

Cadmium burdens in digestive glands (Fig. 1) and adductor muscle were very tightly related to shell height. There were very tight relationships between the weights of selected tissues and shell heights (Table 2), the relationships again being best described by the log–log relationships. Cadmium burden in both adductor muscle and digestive gland increased at an accelerating rate with size to yield the log–log relationships given in Table 2. There were no demonstrable differences in cadmium concentration or burden in the digestive gland between male and female scallops.

Cadmium concentrations in adductor muscle were not related to cadmium concentrations in the digestive gland. The significant relationship between the cadmium burden in the adductor muscle and the cadmium burden in the digestive gland is probably due to the very significant relationship present between the weights of the two tissues. The ratios of either cadmium concentrations or burdens in the digestive gland to the equivalent cadmium value in the adductor muscle were not related to shell height.

We have investigated two other factors (age and total wet weight) in attempting to determine the best relationship between cadmium levels and size. Scallop shells from Georges Bank were aged by two technicians experienced in the art of ageing scallops. Also, the apparent growth rings were counted on the right valve. Cadmium burden in the digestive gland was highly correlated with both age and the number of apparent growth rings. Unfortunately, the age distribution supplied by the two technicians did not agree satisfactorily. There was an average difference of 0.25 ± 0.69 yr between the two sets of data for the Georges Bank scallop shells with negative differences in scallops of less than 90 mm shell height and positive differences for scallops of greater than 90 mm shell height. These results are not necessarily surprising, since scallops on Georges Bank, being exposed to a more consistent climate, do not show the pronounced winter growth interruptions characterizing scallops from other areas (G. Robert, Fisheries Research Branch, P.O. Box 550, Halifax, N.S. B3J 2S7, pers. comm.). These observations plus the relatively large impact of an error of one unit of either age or apparent growth ring number upon the regression analysis compared with the use of shell height suggest that shell height is the appropriate size factor to use in investigating the effect of animal size on the amount of cadmium in the digestive gland. We recommend it as the major biological variable to control in studies such as this.

The total wet weight of the whole animal was also an excellent predictor of the total amount of cadmium in the digestive gland (Table 2). However, we do not recommend its use because of difficulties in determining weight in the field and the real possibility that slightly debilitated or damaged scallops

TABLE 2. Regression relationships between tissue weights (wt, g) tissue cadmium values, shell heights (SH, mm), and age for scallops from Georges Bank (N = 57–60) and Passamaquoddy Bay (N = 35–36). [CD] = mg cadmium/kg wet wt; Cd_T = tissue cadmium burden (μg); DG and AM refer to digestive gland and adductor muscle. Linear, semi-log, and log–log relationships were investigated. Only the best significant relationship is given.

Relationship	Sample site	Equation	Coefficient of determination
$[Cd]_{AM}$ vs. SH	Georges Bank	Not significant	—
	Passamaquoddy Bay	Not significant	—
$[Cd]_{DG}$ vs. SH	Georges Bank	$\log [Cd]_{DG} = -0.015 + 1.049 \log SH$	0.726
	Passamaquoddy Bay	$\log [Cd]_{DG} = 0.287 + 0.796 \log SH$	0.694
Cd_{TAM} vs. SH	Georges Bank	$\log Cd\ TAM = -0.429 + 3.306 \log SH$	0.850
	Passamaquoddy Bay	$\log Cd\ TAM = -6.979 + 3.618 \log SH$	0.923
Cd_{TDG} vs. SH	Georges Bank	$\log Cd\ TDG = -4.984 + 3.776 \log SH$	0.978
	Passamaquoddy Bay	$\log Cd\ TDG = -5.098 + 3.680 \log SH$	0.976
Cd_{TDG} vs. total wt	Georges Bank	$\log Cd\ TDG = -1.1917 + 1.245 \log total\ wt$	0.982
	Passamaquoddy Bay	$\log Cd\ TDG = -0.3912 + 1.206 \log total\ wt$	0.972
Wt ST[a] vs. SH	Georges Bank	$\log wt\ ST = 4.412 + 2.984 \log SH$	0.984
	Passamaquoddy Bay	$\log wt\ ST = 4.563 + 3.117 \log SH$	0.987
Wt AM vs. SH	Georges Bank	$\log wt\ AM = 4.724 + 2.965 \log SH$	0.967
	Passamaquoddy Bay	$\log wt\ AM = 5.006 + 3.091 \log SH$	0.964
Wt DG vs. SH	Georges Bank	$\log wt\ EG = -5.023 + 2.739 \log SH$	0.971
	Passamaquoddy Bay	$\log wt\ DG = -5.381 + 2.882 \log SH$	0.978
$[Cd]_{AM}$ vs. $[Cd]_{DG}$	Georges Bank	Not significant	—
	Passamaquoddy Bay	Not significant	—
Cd_{TAM} vs. Cd_{TDG}	Georges Bank	$\log Cd_{TAM} = -2.046 + 0.878 \log Cd_{TDG}$	0.865
	Passamaquoddy Bay	$\log Cd_{TAM} = -1.959 + 0.972 \log Cd_{TDG}$	0.924
Wt AM vs. wt DG	Georges Bank	$\log wt\ AM = 0.770 + 1.063 \log wt\ DG$	0.966
	Passamaquoddy Bay	$\log wt\ AM = 0.716 + 1.065 \log wt\ DG$	0.977
$\frac{[Cd]_{DG}}{[Cd]_{AM}}$ vs. SH	Georges Bank	Not significant	—
	Passamaquoddy Bay	Not significant	—
$\frac{Cd_{TDG}}{Cd_{TAM}}$ vs. SH	Georges Bank	Not significant	—
	Passamaquoddy Bay	Not significant	—
Cd_{TDG} vs. apparent growth ring number[b]	Georges Bank	$\log Cd_{TDG} = -1.009 + 3.699 \log N$	0.882
Cd_{TDG} vs. age (1)[c]	Georges Bank	$\log Cd_{TDG} = -0.330 + 3.998 \log age\ (1)$	0.985
Cd_{TDG} vs. age (2)[c]	Georges Bank	$\log Cd_{TDG} = 0.820 + 2.356 \log age\ (2)$	0.934
Cd_{TAM} vs. apparent growth ring number[b]	Georges Bank	$\log Cd_{TAM} = 3.000 + 3.247 \log N$	0.770

[a]Soft tissue weight (adductor muscle + digestive gland + residual soft tissue parts).
[b]Number observed on upper valve exterior.
[c]Age in years using two ageing specialists (1 and 2) − average age difference = 0.25 ± 0.69 yr with negative differences in scallop <90 mm shell height and positive differences in scallops >90 mm shell height.

could lose intervalve water during handling.

Fluid losses during dissection were investigated. The fluid lost from each animal was estimated by subtracting shell weight from total wet weight to give a soft tissue weight and subtracting the dissected weights of the adductor muscle, digestive gland, and residual tissues. The resulting value was divided by the soft tissue weight and multiplied by 100 to express the fluid loss as a percentage of soft tissue weight. Georges Bank scallops lost an average of 31.81 ± 3.48% fluid during dissection, while scallops from Passamaquoddy Bay lost 33.32 ± 4.67%. No cadmium was detected in this fluid. Fluid loss (percentage) was not related to size.

Comparison of Cadmium Levels in Scallops from Different Areas

The excellent relationships between cadmium burden in the digestive gland and shell height can be used to compare differences in cadmium burdens between the two sample sites. The simplest way is to plot the ratio of predicted digestive gland cadmium burdens for the two areas for scallops of the same shell height (Fig. 2). As shell height increased the ratio also increased, although at a decreasing rate. Therefore it is important to compare similarly sized scallops from different areas and that the size selected be as large as feasible to minimize the effect of size on the ratio of cadmium burdens. For example, use of scallops of 90–110 mm shell height would result in an increase of less than 5% in the ratio over this size range.

Following these observations, we investigated cadmium levels in scallops (~100 mm shell height) from Georges Bank, Browns Bank, Passamaquoddy Bay, and two areas of Chaleur Bay (Tables 3 and 4). Surprisingly, the amount of cadmium in the digestive glands of scallops from Chaleur Bay was less than that in Georges Bank and Passamaquoddy Bay scallops. Even more surprising, cadmium burden in digestive gland of scallops from Browns Bank was almost five times greater than that in

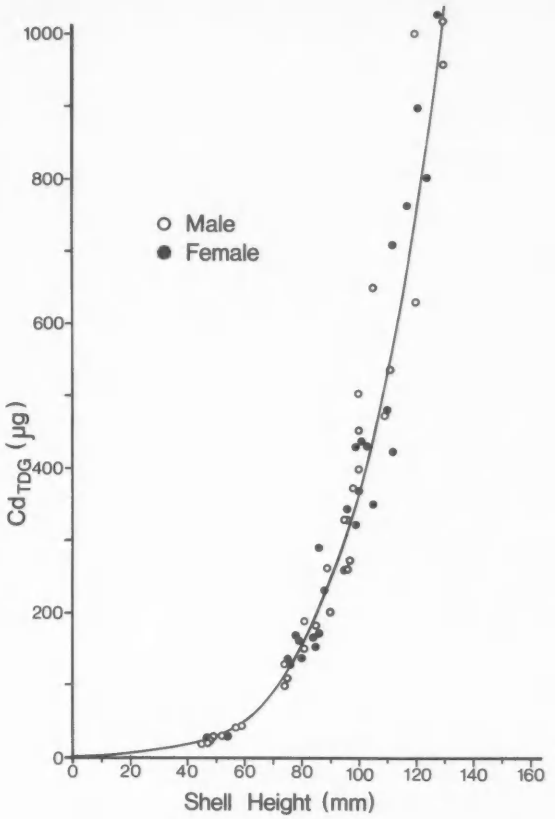

FIG. 1. Relationship between the digestive gland cadmium burden and scallop shell height for Georges Bank.

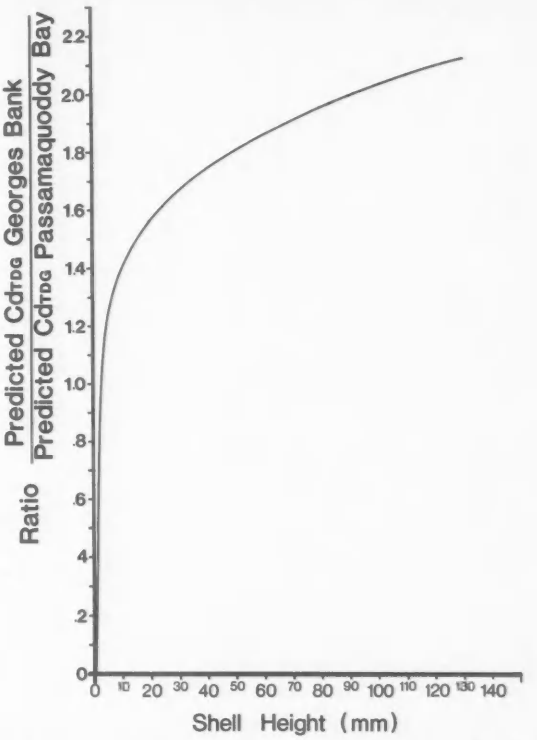

FIG. 2. Relationship between the ratio of cadmium burdens in the digestive glands of Georges Bank scallops and Passamaquoddy Bay scallops to shell height. The relationship was developed from the regression equations for each site.

Chaleur Bay scallops.

Adductor muscle cadmium burdens ranked in a different order. Scallops from Chaleur Bay and Browns Bank had greater adductor muscle burdens than did scallops from Georges Bank and Passamaquoddy Bay. Cadmium concentrations presented a similar picture. Neither ranking could be reconciled simply with known cadmium inputs to the areas.

The ratio between either concentrations or burdens of cadmium in the digestive gland divided by their corresponding adductor muscle value gave a different picture. The ratios of cadmium in the digestive gland to cadmium in the adductor muscle, either as concentration or burden, suggested that contaminated scallops may be recognized by a decreased ratio value (from approximately 850 to 230 in the case of cadmium concentrations in scallops from Georges Bank and Browns Bank compared with Chaleur Bay scallops). The low ratios characterizing Chaleur Bay scallops are not surprising in scallops being exposed to cadmium if the cadmium deposition rate in the digestive gland is less than cadmium uptake by the animal. The ratio was not related to shell height for Georges Bank or Passamaquoddy Bay scallops.

Effect of Starvation on Scallop Tissue Masses and Cadmium Levels

To investigate more fully the distribution of cadmium in scallop soft tissues and the effect of external and internal factors on both distributions and levels of cadmium, scallops from Georges Bank were held without feeding for approximately 14 mo in our aquarium in filtered seawater (cadmium = 0.030 mg/L) prior to dissection and cadmium determinations (Table 4). Many of the animals discharged reproductive products during the holding period (June–August). Starvation did not affect the total amount of cadmium present in the soft tissues of the scallops although the concentrations in the digestive gland and the adductor muscle increased significantly as the organs lost weight. The total weight of the animals and the shell weight was not affected but the weights of the various soft tissues decreased significantly (Table 3). There was a significant increase in the cadmium burden of the adductor muscle of the starved scallops from 1.93 ± 0.54 mg/kg to 3.25 ± 0.93 mg/kg ($P < 0.05$). The source of cadmium to the muscle is obscure. It may have come from the water in which the scallops were held, adventitious food in the water supply, or from other tissues. The cadmium burdens of the digestive gland and residual soft tissues did not decrease significantly. The cadmium increase in the adductor muscle after starvation is such a small fraction of the total cadmium present in the animal, however, that this small loss from either digestive gland or other tissues would be undetected. Interestingly, the ratios of digestive gland cadmium to that of the adductor muscle were 287 for the concentrations and 95 for the burden,

TABLE 3. Mean ± SD total animal and tissue weights (g) and intertissue weight factors in
of whole animal weight. ns and s indicate not significant and significant ($P < 0.05$) bet

Location	N	Whole animal	Shell	Shell contents[a]	
Chaleur Bay					
Petit Rocher (47°47′N, 65°55′W)	6	101.0±0.8	55.88±0.17 (55.3)	45.14±5.42	8
Belledune Harbour (47°55′N, 65°35′W)	10	128.4±0.8	71.07±0.06 (55.4)	53.56±8.33	1
Browns Bank (42°29′N, 65°46′W)	10	153.3±8.6	107.30±0.47 (70.0)	46.02±3.72	
Passamaquoddy Bay (45°05′N, 64°57′W)	6	179.1±4.1	124.09±5.13 (69.3)	55.04±15.04	1
Georges Bank (42°04′N, 66°47′W)	6	145.0±7.3	81.04±5.38 (55.9)	64.01±5.09	1
Georges Bank (held live for 14 mo in aquarium)	10	146.0±21.9 ns	91.04±7.44 (62.4) ns	55.00±7.45	

[a]Whole animal weight minus shell weight.
[b]Weight of tissues remaining after removal of adductor muscle and digestive gland.
[c]Sum of weights of tissue residue, adductor muscle, and digestive gland.

tors in 100-mm-sized scallops from various locations. Value in parentheses is mean percemt
95) between original and starved scallops.

	Adductor muscle	Digestive gland	Tissue residue[b]	Dissected tissues[c]	Dissected tissues/ shell contents
2	8.18±1.01	2.15±0.17	16.17±2.51	26.51±2.72	0.588±0.020
3	12.71±0.90	2.74±0.67	—	—	—
2	7.33±0.79	2.72±0.30	20.58±3.15	30.64±3.16	0.669±0.007
04	14.23±0.81	2.83±0.52	18.92±0.37	35.98±9.19	0.673±0.007
9	16.68±1.55	3.40±0.40	23.47±3.36	43.55±4.46	0.683±0.027
5	4.46±1.54 s	1.45±0.27 s	13.37±1.19 s	19.42±2.57	0.353±0.009

and.

TABLE 4. Mean cadmium values in 100-mm-sized scallop tissues from various locations within the Maritime area. ns and s indicate not significant and significant ($P < 0.05$) differences between original and starved scallops.

Location	mg Cd/kg wet wt			Tissue burden (μg Cd)			
	Adductor muscle	Digestive gland[a]	Soft tissue residue	Adductor muscle	Digestive gland	Soft tissue residue[a]	Total burden
Chaleur Bay							
Petit Rocher	0.348±0.119	78.38±5.27 (225)	1.51±0.33	2.82±0.89	166.3±1.4 (59)	24.28±0.80	191.4±4.8
Belledune Harbour	0.257±0.066	61.15±1.72 (238)	—	3.30±0.05	164.5±8.9 (50)	—	—
Browns Bank	0.338±0.125	291.3±4.7 (862)	—	2.46±0.90	789.7±19.3 (321)	—	—
Passamaquoddy Bay	0.121±0.224	69.41±2.41 (574)	—	1.70±0.36	183.8±0.7 (108)	—	—
Georges Bank	0.121±0.033	98.25±0.32 (812)	1.08±0.44	1.93±0.54	313.8±5.3 (163)	24.81±0.97	340.4±3.2
Georges Bank (held live for 14 mo)	0.756±0.257 s	217.7±3.0 s (288)	1.90±0.79 ns	3.25±0.93	307.3±6.5 ns (95)	25.95±0.07 ns	336.5±8.7 ns

[a]Ratio of mean digestive gland Cd to mean adductor muscle Cd for concentration and tissue burden.

values nearer to those characterizing Chaleur Bay scallops than to those for scallops from the other three sample areas. This is not surprising, since one would expect that catabolism associated with starvation would tend to free cadmium from its protein complex(es) as the animal metabolizes its own tissues to supply essential nutrients. Under starvation conditions or under conditions of excessive input of cadmium such as in a contaminated environment, cadmium-binding proteins synthesized in the digestive gland could be less than the amount of protein required to bind all of the free cadmium, allowing excess cadmium to "spill over" into other tissues such as the adductor muscle.

The starvation experiment also suggests an explanation for the cadmium concentrations and burdens observed in wild scallops. Although scallops of essentially the same size (shell height) were selected from each area, the mean total weights varied substantially (101.0−179.1 g) with mean total weights decreasing in the order Passamaquoddy Bay, Browns Bank, Georges Bank, Belledune Harbour (Chaleur Bay), and Petit Rocher (Chaleur Bay). The major source of these differences in weight was the weights of the shells (Table 3). The range in mean dissected tissue weights (Table 3), however, was 26.50−43.42 g, increasing in the order Petit Rocher, Browns Bank, Passamaquoddy Bay, and Georges Bank. Starvation decreased both the absolute weight of the adductor muscle (by a factor of almost four) and its weight relative to the other soft parts. Starvation caused a less drastic decrease in digestive gland weight, not surprising since muscle wastage in starvation is generally greater than that of critical organs.

Analysis of the tissue weights of scallops from Georges Bank and Passamaquoddy Bay used in the size study described above showed for each population that the adductor muscle maintained a constant weight percentage of the shell contents, regardless of animal size (shell height), with a mean of 24.00 ± 2.91% for Georges Bank scallops and 28.02 ± 3.61% for Passamaquoddy Bay scallops. Adductor muscles from Browns Bank scallops were smaller, averaging only 16.0% of the shell contents. Starved Georges Bank scallop averaged 8.1% adductor muscle compared with 26.1% immediately after capture (100 mm shell height).

In the case of scallops from Browns Bank, the digestive gland − adductor muscle cadmium ratios are very high, suggesting that these scallops sequester most of the cadmium in their digestive glands. Seawater cadmium concentrations in the area are estimated to be around 30 ng/L (J. M. Bewers, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, pers. comm.), a low value characteristic of offshore water. Sediment cadmium levels should also be very low, since cadmium concentrates on fine material (Loring 1984) and scallops do not thrive on very fine sediments, preferring sand and gravel. The inference is that food is the source of the very high cadmium burdens and concentrations in Browns Bank scallops.

We believe the Browns Bank results indicate scallops feeding on a nutritionally marginal diet containing relatively large amounts of cadmium. The small adductor muscle (as compared with other scallops) also supports a picture of marginal nutrition. Nutritional considerations would also play a role in the other sample areas but we do not have sufficient information to comment on them. It is, of course, possible that the Browns Bank results are due to nonnutritional factors such as genetics but we were unable to find published information to support such nonnutritional causes.

Overall, the results clearly demonstrate that use of tissue concentrations or burdens of contaminants alone as an indicator of trace metal contamination can be misleading, since tissue levels are drastically affected by important non-pollution-related factors such as food supply. Intertissue ratios, i.e. the ratio of the concentration of cadmium in the digestive gland to that in the adductor muscle or the corresponding burden ratios, appear useful for ranking areas in terms of cadmium inputs.

Further studies are needed to set a firm foundation for estimating relative chemical contamination levels within various scallop populations. Similar investigations with mussels and other bivalves will sharpen our understanding of such animals in programs such as the "Mussel Watch" (Goldberg 1975).

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Acclimation to Copper by Rainbow Trout, *Salmo gairdneri*: Physiology

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Laurén, D. J., and D. G. McDonald. 1987. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can. J. Fish. Aquat. Sci. 44: 99–104.

Juvenile rainbow trout (*Salmo gairdneri*) were exposed to 55 μg copper·L^{-1} for 28 d and then transferred to uncontaminated water for 7 d. Whole body sodium concentration and sodium uptake (J_{in}) were measured at weekly intervals; J_{in} was measured at various Na^+ concentrations and kinetic parameters estimated. After 24 h of copper exposure, the maximum rate of sodium uptake (J_{max}) was inhibited by 55%, the affinity for sodium (K_m) reduced by 49%, and whole body Na^+ decreased by about 12.5%. After 7 d of exposure, whole body Na^+ had returned to control values, but J_{max} was still inhibited by 41%. Recovery of whole body Na^+ occurred largely by a reduction of sodium efflux (J_{out}). Both J_{max} and K_m continued to recover until day 28, at which time J_{in} had returned to control values. We conclude that acclimation to sublethal copper depends on changes in both Na^+ transport and permeability.

De jeunes truites arc-en-ciel (*Salmo gairdneri*) ont été exposées pendant 28 jours à 55 μg·L^{-1} de cuivre puis transférées dans une eau non contaminée pendant 7 j. On a mesuré à toutes les semaines la concentration et l'absorption de sodium (J_{in}) dans tout l'organisme; on a mesuré la valeur J_{in} à diverses concentrations de Na^+ et estimé les paramètres cinétiques. Après 24 h d'exposition au cuivre, le taux maximal d'absorption de sodium (J_{max}) a diminué de 55 %, l'affinité pour le sodium (K_m) a été réduite de 49 % et le Na^+ de tout le corps a diminué d'environ 12,5 %. Après 7 j d'exposition, le Na^+ de tout le corps était revenu aux valeurs des témoins, mais la valeur de J_{max} était encore réduite de 41 %. Il y a eu rétablissement de Na^+ dans tout le corps grâce dans une large mesure à une réduction de la sortie de sodium (J_{out}). Les valeurs J_{max} et K_m ont continué à se rétalir jusqu'au 28^e jour, moment auquel J_{in} était revenu aux valeurs des témoins. Nous concluons que l'acclimatation à une dose sublétale de cuivre dépend de changements dans le transport des ions Na^+ et dans la perméabilité.

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Although copper, zinc, cadmium, and mercury can be acutely toxic to fish, numerous laboratory studies have shown that prior sublethal exposure to metals can result in enhanced lethal resistance (Chapman 1978; Bouquegneau 1979; Pascoe and Beattie 1979; Dixon and Sprague 1981; Buckley et al. 1982; Duncan and Klaverkamp 1983). Fewer studies have demonstrated true acclimation of fish to metals, especially if one accepts that acclimation involves the return of normal rate functions to the preexposure steady state, during the continued presence of the stressor (Prosser 1973). Acclimation to copper was indicated by McKim et al. (1970), who showed the recovery of plasma chloride to normal values after 331 d of exposure to 38 μg copper·L^{-1}, and by Lett et al. (1976), who found that growth rate approached control values within 40 d of exposure to 225 μg copper·L^{-1} (in very hard, high-alkalinity water). However, very little is known about the physiological basis for this apparent acclimation. Thus, the objectives of the present study were to determine if true acclimation to copper occurs in the laboratory and to describe some of the physiological changes which occur during acclimation.

Recently, we demonstrated that the primary site of sublethal copper toxicity to rainbow trout (*Salmo gairdneri*) is the sodium transport mechanism of the gills (Laurén and McDonald 1985, 1986). Many authors have shown that sodium uptake may be described by Michaelis–Menten kinetics (Kerstetter et al. 1970; Maetz 1972; DeRenzis and Maetz 1973) and thus may be analysed in terms of saturability (J_{max}) and affinity (K_m) for substrate. If trout are able to acclimate to copper, this acclimation should occur at the primary site of toxic insult, the gills, and should be apparent in terms of velocity and/or affinity of sodium uptake. To test this hypothesis, we exposed juvenile rainbow trout to a sublethal concentration of copper for 28 d and analysed sodium uptake kinetics at regular intervals. In a companion paper, we describe some of the biochemical changes which occur during acclimation to copper (Laurén and McDonald 1987).

Methods and Materials

Two different series of experiments were conducted. The first type assessed the effects of long-term copper exposure on sodium uptake kinetics and whole body sodium concentration. To assure reproducibility, this experiment was repeated. The second series of experiments examined the effects of short-term copper exposures on sodium uptake and whole body sodium concentration.

Juvenile rainbow trout (mean = 2.77 ± 0.22 SEM, range =

1.05–5.71 g wet wt, $n = 490$) were obtained from Spring Valley Trout Farm, Petersburg, Ontario, and maintained in flowing dechlorinated natural hardwater (pH = 7.8 ± 0.1, $[Na^+] = 595 \pm 25$ μ*M*, $[Cl^-] = 890 \pm 34$ μ*M*, $[Ca^{2+}] = 1040 \pm 51$ μ*M*, [ammonia] = 28 ± 7 μ*M*, and [titratable alkalinity] = 875 ± 25 μ*M* (fixed endpoint titration to pH 4.0), $n = 8$). In the first type of experiment the fish were exposed to copper in a flow-through system for 28 d and allowed to recover in the absence of copper for 7 d. The fish were fed daily for the entire period of exposure and recovery. The experiments were conducted at 15 ± 1°C with a daily photoperiod of 14 h light and 10 h darkness. Copper stock (as $Cu(NO_3)_2$) was metered by a peristaltic pump into diluent water in a head tank. This tank, in turn, fed eight separate 80-L tanks at a rate of 75 mL·min^{-1} each. Each tank was aerated and contained 40 fish. Under these conditions each tank received about twice the minimum daily volume replacement recommended by Craig and Beggs (1979) for copper. The copper concentrations in the fish tanks were measured daily and averaged 54.9 ± 1.7 μg·L^{-1} ($n = 54$).

Sodium Uptake Kinetics

Long-term copper exposures

At weekly intervals, a subsample of fish from the flow-through copper exposure was transferred to individual flux chambers. To measure Na^+ uptake kinetics the fish were exposed for 2 h to one of seven sodium concentrations (approximately 25, 50, 100, 200, 400, 600, or 800 μ*M* Na^+, labelled with 148 KBq of $^{22}Na \cdot L^{-1}$) made by adding NaCl to deionized water. Five fish were exposed at each of the sodium concentrations, and copper (55 μg·L^{-1} as $Cu(NO_3)_2$) and calcium (1000 μ*M* as $Ca(NO_3)_2$) were held constant. On day 0, before the exposure began, and on day 1 and 7 following the end of copper exposure, fish were exposed for 2 h to the same conditions as above, but without copper. Unidirectional sodium flux measurements were conducted in covered, aerated, 500-mL polyethylene containers each holding a single fish and filled with 250 mL of medium. Sodium influx (J_{in}) was calculated from measurements of whole body ^{22}Na according to the following formula:

$$(1) \quad J_{in} = \frac{WBA}{SA \times W \times t}$$

where SA is the mean specific activity (counts per minute per micromole) of the exposure media for the 2-h flux period, WBA is the whole body activity (counts per minute), *W* is the wet weight of the fish to the nearest 0.01 g, and *t* is 2 h. Radioactivity of the water and fish was measured in a Nuclear Chicago well-type gamma counter.

Prior to flux measurements, each fish was transferred to and held for 30 min in a 250-mL rinse bath and then transferred to the flux chamber. This allowed the fish to adjust to confinement in the flux chamber and also reduced the carryover of ions from the exposure system. The rinse water was identical in composition to the flux chamber water. During flux measurements, water samples were collected at time 0 (prior to the addition of fish) and at 60 and 120 min. The fish were then killed by cervical dislocation, weighed, and counted in a gamma counter.

Following gamma counting, the fish were digested in 5 mL of concentrated nitric acid. These digests, as well as the water samples collected during the experiment, were analysed for Na^+, K^+, Ca^{2+}, and Cu^{2+} with a Varian 1275 atomic absorption spectrophotometer. Lanthanum chloride was added to Ca^{2+} samples to reduce the interference due to the high Na^+ levels.

Short-term copper exposures

In this series, juvenile trout were first exposed to 100 μg copper·L^{-1} for 24 h in artificial hardwater (200 μ*M* NaCl, 1000 μ*M* $Ca(NO_3)_2$, pH 7.8) and then exposed for an additional 24 h to 0, 50, 100, or 200 μg copper·L^{-1}. At the end of this period the fish were killed and analysed for whole body Na^+ as described above. $J_{in}^{Na^+}$ was measured as described above. Based on whole body sodium levels, sodium efflux constants were calculated using the exponential decay equation

$$(2) \quad [Na]_{t24} = [Na]_{t0} \times e^{-dt}$$

which can be rearranged as

$$(3) \quad d = \frac{(\ln[Na]_{t0}) - (\ln[Na]_{t24})}{t}$$

where $[Na]_{t0}$ is the mean whole body $[Na^+]$ per unit weight at the beginning of a 24-h period of exposure, $[Na]_{t24}$ is the whole body $[Na^+]$ at the end of a 24-h period, *t* is 24 h, and *d* is the sodium efflux constant. This is a dimensionless value which estimates diffusional efflux, independent of the Na^+ gradient, providing certain conditions are met (see Results).

Means ± 1 standard error of the mean were calculated. Significance at $P \leq 0.05$ was tested by Student's two-tailed *t*-test for unpaired treatments. Lineweaver–Burk transformed data (i.e. $1000/J_{in}$ vs. $1000/[Na^+]$) were evaluated by least squares linear regression and the resulting equations used to estimate the kinetic parameters, J_{max} and K_m, where $1000/J_{max}$ = the *x*-intercept and $1000/K_m$ = the *y*-intercept.

Results

To ensure the reproducibility of the results, the uptake kinetics experiment was run on two separate occasions. The first experiment was begun on February 20, 1985, and the replicate experiment was begun on April 16, 1985. There were no significant differences between the results of the two experiments in regard to water copper concentration, sodium uptake, or whole body ions. Therefore the data were pooled for analysis.

Sodium Influx

In control fish, sodium influx (J_{in}) exhibited apparent saturation kinetics (day 0, Fig. 1). Although the gill is a complex organ, and the nature and location of the sodium transport mechanism is not known, $J_{in}^{Na^+}$ could be effectively described using the Lineweaver–Burk procedure for estimating kinetic parameters (i.e. J_{max} (Fig. 2a) and K_m (Fig. 2b)). For control fish, this yielded a maximal uptake velocity (J_{max}) of 540 ± 37 nmol·g^{-1}·h^{-1} and an apparent affinity for substrate (K_m) of 57 ± 4 μ*M*.

After 24 h of exposure to 55 μg copper·L^{-1}, there was significant inhibition of J_{in} at all Na^+ concentrations relative to control values (day 1, Fig. 1). The apparent J_{max} and K_m were calculated as 244 ± 28 nmol·g^{-1}·h^{-1} and 118 ± 2 μ*M*, respectively (Fig. 2a, 2b), representing a 55% reduction in maximal velocity and a 49% reduction in apparent affinity.

Following 7 d of copper exposure, J_{max} had recovered relative to day 1, but was still only about 59% that of control values (Fig. 2a). After 14 d, J_{max} was about 77% that of controls, a significant increase over the day 7 value (Fig. 2a). The appar-

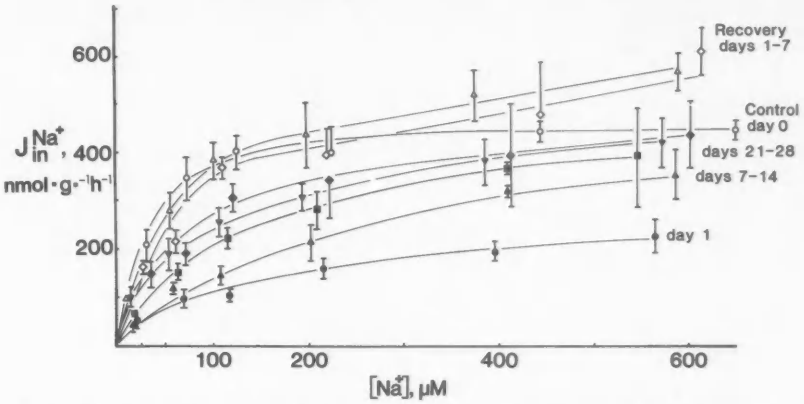

FIG. 1. Inhibition and recovery of sodium uptake in juvenile rainbow trout exposed to 55 μg copper·L^{-1} in natural hardwater at neutral pH. Each point represents the mean ± 1 SEM of 10 fish. ○, preexposure controls; ●, day 1 of copper exposure; ▲, day 7; ■, day 14; ▼, day 21; ◆, day 28; △, recovery day 1; ◇, recovery day 7.

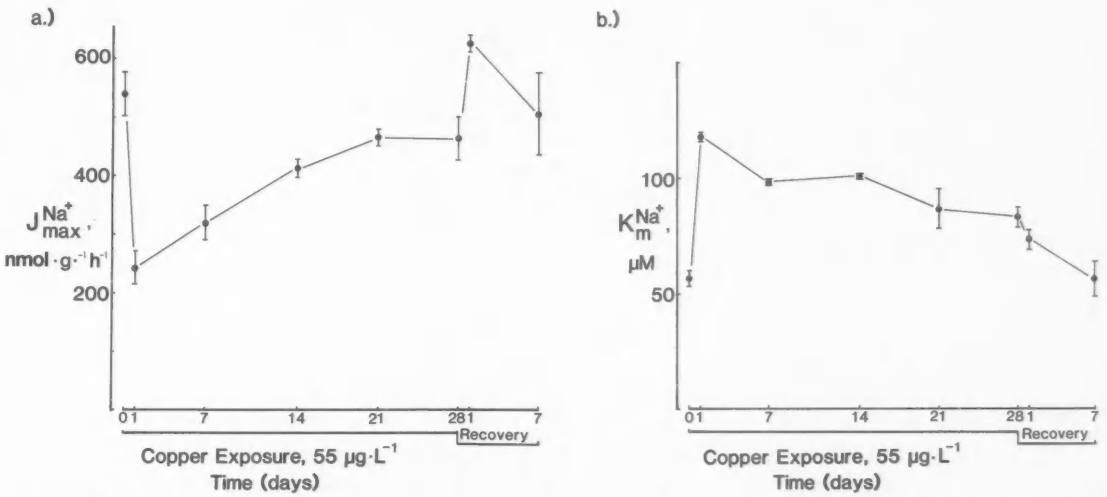

FIG. 2. (a) Maximal velocity (J_{max}) calculated by linear regression of Lineweaver–Burk transformed data in the form $1000/J_{in}$ vs. $1000/[Na^+]$, $n = 60$, (b) apparent affinity (K_m) calculated as in Fig. 2a.

ent affinity of the carrier was estimated to be 98 ± 0.1 μ*M* on day 7 and 101 ± 0.7 μ*M* Na^+ on day 14 (Fig. 2b). Thus, K_m was still inhibited by about 42% relative to controls.

A general trend for the continuing recovery of J_{in} was found from day 14 to 28, but was significant only at about 25 μ*M* Na^+ on day 21 and at 25 and 100 μ*M* Na^+ on day 28 (Fig. 1). No significant changes were found between days 21 and 28. On day 28, J_{max} was still inhibited by about 14% (Fig. 2a), but this was not significantly different from control values, whereas K_m was still significantly inhibited by about 31% relative to control values (Fig. 2b).

After 28 d the fish were transferred to natural tap dechlorinated hardwater (i.e. ambient copper was less than 5 μg·L^{-1}). Within 24 h of recovery, no significant differences from control values of $J_{in}^{Na^+}$ were found except at 600 μ*M* (R1, Fig. 1) where J_{in} was significantly higher than found under control conditions. J_{max} was calculated to be 630 ± 15 nmol·g^{-1}·h^{-1} (Fig. 2a), but K_m was still inhibited by about 23% (Fig. 2b). After 7 d of recovery in copper-free water, J_{in} at 600 μ*M* Na^+ was still significantly greater than under preexposure control conditions (R7, Fig 1). Despite this difference, J_{max} was 510 ± 71 nmol·g^{-1}·h^{-1} and K_m was 57 ± 8 μ*M*. These values were nearly identical with those of controls found on day 0 (i.e. 540 ± 37 nmol·g^{-1}·h^{-1} and 57 ± 4 μ*M*; Fig. 2a, 2b).

Whole-Body Ions

In control fish in natural dechlorinated hardwater, whole body Na^+, K^+, and Ca^{2+} concentrations were 56.9 ± 1.2, 98.9 ± 2.8, and 78.5 ± 2.2 μmol·g wet wt^{-1}, respectively (Table 1).

Within 24 h of exposure to copper, whole body Na^+, K^+,

TABLE 1. Whole body ions in juvenile rainbow trout exposed for 28 d to 55 μg copper·L^{-1} and 7 d of recovery in copper-free water. For the exposure period, each value is the mean ± 1 SEM of 60 fish; for the recovery period, each value is the mean ± 1 SEM of 30 fish. Asterisks indicate a significant ($P \leq 0.05$) decrease from control (day 0) values.

	Wet weight (g)	$[Na^+]$ (μmol·g^{-1})	$[K^+]$ (μmol·g^{-1})	$[Ca^{2+}]$ (μmol·g^{-1}
Exposure				
Day 0	2.42±0.12	56.9+1.2	98.9±2.8	78.5±2.3
Day 1	2.22±0.09	49.8±0.6*	93.9±0.7*	74.8±1.1*
Day 7	2.38±0.11	56.6±1.6	102.0±5.1	79.5±2.8
Day 14	2.64±0.11	57.7±0.9	104.4±4.0	81.1±1.4
Day 21	2.82±0.13	58.3±0.7	106.8±4.3	79.2±2.7
Day 28	3.34±0.12	56.4±0.7	107.9±4.7	79.2±1.5
Recovery				
Day 1	3.31±0.11	57.5±0.8	111.5±5.7	79.9±0.9
Day 7	3.78±0.16	57.5±0.6	113.8±7.5	79.3±1.1

and Ca^{2+} concentrations decreased significantly to 49.8 ± 0.6, 93.9 ± 0.7, and 74.8 ± 1.1 μmol·g^{-1}, respectively (Table 1). For Na^+, this amounted to an average net loss rate of about 295 nmol·g^{-1}·h^{-1}, or about 12.5% of the whole body Na^+ content.

After 7 d of copper exposure, whole body Na^+, K^+, and Ca^{2+} concentrations had recovered completely (Table 1), and these values were maintained for the duration of the experiment. Whole body K^+ increased significantly over the control value by day 14 and continued to increase as body weight increased. Body weight and K^+ concentration were significantly correlated ($r^2 = 0.8813$).

Short-Term Copper Exposure

In a previous study, we showed that copper exposure leads to concentration-dependent decreases in both $J_{in}^{Na^+}$ and whole body Na^+ in juvenile trout (solid lines, Fig. 3a and 3b; Laurén and McDonald 1986). In the present study, we have shown that trout preexposed to 100 μg copper·L^{-1} for 24 h and then returned to copper-free water suffered comparable losses of whole body Na^+, although J_{in} recovered (0 μg copper·L^{-1}, broken lines, Fig. 3a and 3b). When preexposed fish were reexposed to 50−200 μg copper·L^{-1} for a second 24 h, they again suffered losses of whole body Na^+, but at a lower rate than fish exposed for the first time (Fig. 3b). However, much of this decrease in loss was likely due to the reduction in the Na^+ diffusion gradient arising from copper preexposure. To account for this factor, the exponential decay equation was used to calculate Na^+ efflux constants, independent of the Na^+ gradient (see Methods and Materials). The use of this equation is only justified when J_{in} approaches zero (i.e. J_{net} is entirely due to J_{out}). In the present series of experiments, this condition was only met in those fish exposed to 200 μg copper·L^{-1} (Fig. 3a). The efflux constant calculated for fish exposed for a single 24-h period was 0.0325, while that calculated for the preexposed group was 0.0154. This is a reduction in the Na^+ efflux constant of 53%, and suggests a major reduction in Na^+ permeability in the preexposed group.

Discussion

The objective of the present study was to examine the physiological basis for acclimation of fish to sublethal levels of copper. In previous studies with rainbow trout, we have shown that one of the major mechanisms of copper toxicity to fish is the disruption of normal gill ionoregulatory function. Copper concentrations as low as 12.5 μg·L^{-1} inhibit sodium uptake and disrupt sodium balance, with death ensuing within 24 h at copper concentrations greater than 100 μg·L^{-1} (Laurén and McDonald 1985, 1986). In the present study, we confirmed that 55 μg copper·L^{-1} inhibits sodium uptake and reduces whole body sodium concentration. In addition, however, we have now shown that both of these parameters can recover completely within 21 d. Furthermore, since naturally occurring surface water copper concentrations generally range from about 5 to 100 μg copper·L^{-1} (Spry et al. 1981), the present results suggest that these same mechanisms may be responsible for acclimation to copper in the wild.

In the present study, whole body Na^+ returned to control levels before J_{in} completely recovered (Table 1; Fig. 1). Because we have shown that the inhibition of J_{in} (at least at 100 μg·L^{-1}) is, in fact, greater at 48 h than at 24 h (Fig. 3a), the recovery of whole body Na^+ is best explained by a decrease in branchial sodium efflux (J_{out}) to levels below that found in normal control fish. Renal compensation is unlikely to be important because the kidney accounts for a maximum of only about 10% of the total sodium efflux in control animals (McDonald and Wood 1981), and copper exposure would be expected to cause renal damage and thereby stimulate rather than reduce renal sodium losses. However, reductions in J_{out} play an important role in ameliorating the effects of low pH (McDonald 1983), lanthanum (Eddy and Bath 1979), and copper (Laurén and McDonald 1985). At a $J_{in}^{Na^+}$ of 244 nmol·g^{-1}·h^{-1} (i.e. J_{in} after 24 h), the net loss of whole body Na^+ found by 24 h could be fully compensated within 7 d if $J_{in}^{Na^+}$ were reduced to about 40% of control values. In fact, we have shown that reductions in J_{out} of up to 50% occur within 12 h in adult trout exposed to copper (Laurén and McDonald 1985).

J_{out} may be reduced by either a decrease in the Na^+ gradient due to ion losses or by a reduction in epithelial permeability. By using the exponential decay equation, we have shown a gradient-independent reduction in Na^+ efflux within 48 h of exposure to copper. The nature of this reduction in permeability is unknown and could be either a Na^+-specific reduction or a more general reduction in the permeability of the branchial epithelium to both cations and anions.

In general, the mechanism by which epithelial permeability is reduced is not presently understood. However, the administration of exogenous mammalian prolactin reduces gill permeability in several fishes (Dharmamba and Maetz 1972; Wendelaar Bonga and Van der Meij 1981), and acid exposure increases the metabolic activity of prolactin cells in the brook trout (Notter et al. 1976). This suggests that the reduction of epithelial permeability may be under endocrine control and may be an essential part of the acclimation of fish to general ionoregulatory disturbances.

According to classical Michaelis−Menton kinetics, a reduction of J_{max} indicates noncompetitive inhibition, while an increase in K_m indicates competitive inhibition. Thus, if it is accepted that sodium uptake occurs via a single branchial enzyme system such as Na^+-K^+-ATPase, copper affects $J_{in}^{Na^+}$ by mixed-type inhibition. However, it must be cautioned that the gill is a very complex tissue and little is known about the factors controlling sodium uptake across either the apical or basolateral cell membranes or, indeed, the number or types of

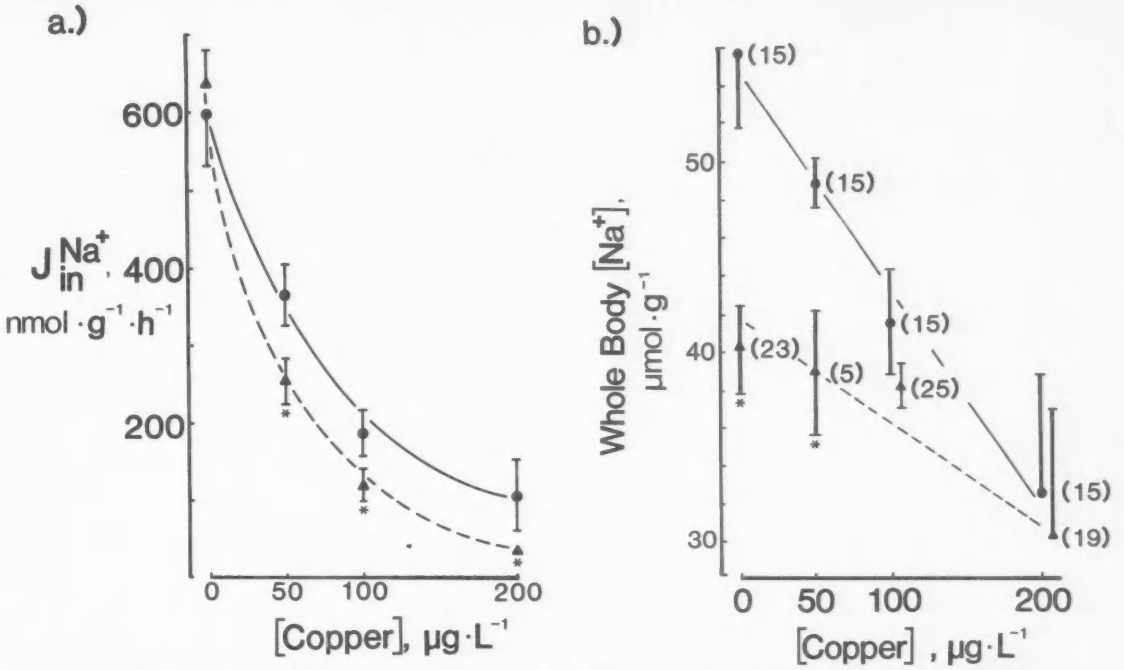

FIG. 3. (a) Effects of copper on sodium uptake (J_{in}) in juvenile rainbow trout exposed for 24 h (●) (data from Laurén and McDonald 1986) or preexposed to 100 µg copper · L^{-1} for 24 h and then exposed for an additional 24 h (▲) (i.e. a total of 48 h of exposure); (b) effects of copper on losses of whole body Na^+ in the same fish as in Fig. 3a. Sample size is indicated in parentheses. Asterisks indicate a significant ($P \leq 0.05$) difference between 24- and 48-h exposures.

transporters which might be found. Nevertheless, Na^+-K^+-ATPase is generally thought to be a major component of the biochemical mechanism for Na^+ transport in all animals, and the replacement of damaged enzyme subunits could result in the recovery of both J_{max} and K_m.

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Acclimation to Copper by Rainbow Trout, *Salmo gairdneri*: Biochemistry

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Whole body, gill, and liver copper uptake, gill Na^+-K^+-ATPase specific activity, and gill and liver acid-soluble thiols (AST), glutathione, and cysteine of rainbow trout (*Salmo gairdneri*) were measured during 28 d of exposure to 55 μg copper·L^{-1}. Na^+-K^+-ATPase specific activity was inhibited by 33% within 24 h of copper exposure, but this was compensated by a significant increase in microsomal protein so that the total Na^+-K^+-ATPase activity per milligram of gill tissue returned to normal by day 14. There was no accumulation of copper and no increase in AST, glutathione, or cysteine in the gill. However, after 7 d of exposure, hepatic AST and glutathione had increased by about 2 times, and a sulfhydryl-rich, acid-soluble protein, tentatively identified as metallothionein, increased by 2.8 times. Copper accumulation was highest in the liver, but other tissues also accumulated copper.

On a mesuré pendant 28 j d'exposition à 55 μg·L^{-1} de cuivre l'absorption de cuivre dans tout le corps, les branchies et le foie, l'activité spécifique de l'ATPase-Na^+-K^+ dans les branchies ainsi que la teneur de thiols solubles dans l'acide (TSA), de glutathion et de cystéine dans les branchies et le foie de truites arc-en-ciel (*Salmo gairdneri*). En moins de 24 h d'exposition au cuivre, l'activité spécifique de l'ATPase-Na^+-K^+ a été réduite de 33 %, mais cela a été contrebalancé par une augmentation importante de protéine microsomiale de sorte que l'activité totale de l'ATPase-Na^+-K^+ par milligramme de tissu branchial est revenue à la normale au 14e jour. Il n'y a pas eu accumulation de cuivre ni augmentation de TSA, de glutathion ou de cystéine dans les branchies. Cependant, après 7 j d'exposition, les TSA et le glutathion du foie ont augmenté de 2 fois environ tandis qu'une protéine soluble dans l'acide, riche en groupes sulfhydryles, identifiée provisoirement comme étant la métallothionéine, a augmenté de 2,8 fois. L'accumulation de cuivre a été la plus élevée dans le foie, mais il y a eu également accumulation dans d'autres tissus.

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Previous biochemical studies of acclimation to copper by fish have shown a correlation between increased resistance and hepatic metallothionein (Dixon and Sprague 1981a, 1981b; Buckley et al. 1982; McCarter et al. 1982). Hepatic metallothionein has been proposed to reduce copper toxicity in fish by sequestering copper away from metabolically important enzyme systems (Dixon and Sprague 1981b; McCarter et al. 1982); in mammals, where metals are acquired across the gut (or injected), such a mechanism appears to explain increased resistance (Cousins 1985). However, we have shown that the gill is the first and, initially at least, the most important target of copper toxicity in fish (Laurén and McDonald 1985, 1986). Furthermore, we have shown that acclimation occurs by the recovery of branchial ionoregulatory function (Laurén and McDonald 1987).

The biochemical basis for this recovery may be an increase in the number of Na^+-K^+-ATPase units, as has been shown in metal-stressed fish by several authors (Bouquegneau 1977; Shephard and Simkiss 1978; Watson and Beamish 1980; Stagg and Shuttleworth 1982), or may be due to the protection of the transport mechanism by metallothionein, as proposed by Bouquegneau et al. (1975). To delineate between these two possibilities, we measured branchial Na^+-K^+-ATPase and branchial metal-binding ligands in fish exposed to 55 μg copper·L^{-1} for 28 d. Because previous authors have suggested a major role of the liver in the detoxification of copper, we also measured hepatic metal-binding ligands and compared the uptake of copper by the liver, gills, and whole body.

Methods and Materials

Juvenile rainbow trout (*Salmo gairdneri*) (2.77 ± 0.22 SEM, range 1.05–5.71 g wet wt, n = 490) were exposed to 55 μg copper·L^{-1} as described in the previous paper (Laurén and McDonald 1987). Subsamples of fish were removed at regular intervals for analyses.

Copper Uptake

At weekly intervals, the net whole body accumulation of copper was determined by atomic absorption (Varian 1275) using the same acid digests used for whole body Na^+ measurements (Laurén and McDonald 1987).

The size of the exchangeable copper pool of the gills, liver, and carcass (i.e. whole body minus gills and liver) was also measured. A separate subsample of 10 fish was removed from the continuous exposure system at weekly intervals and exposed for 24 h to 55 μg copper·L^{-1}, labelled with ^{64}Cu (22 MBq·μmol^{-1}). Sodium and calcium concentrations in the media were held constant at 200 and 1000 μM, respectively. Radioactivity of water, gills, liver, and carcass was measured in a Nuclear Chicago well-type gamma counter. After count-

ing, these tissues were digested in concentrated nitric acid and analysed as above for copper concentration. The portion of the total tissue copper that was exchangeable within 24 h was then estimated as

$$(1)\quad \frac{^{64}\text{Cu} \times (\text{SA})}{\text{Total tissue copper}} \times 100$$

where ^{64}Cu is the counts per minute of radiocopper per gram wet weight taken up by the tissue during 24 h, SA is the specific activity of the exposure water (micrograms per counts per minute), and total tissue copper (micrograms per gram wet weight) was determined by atomic absorption spectrophotometry. Control copper values for gill, liver, and carcass were collected from a separate group of 10 fish that had never been exposed to copper above ambient levels (i.e. less than 5 $\mu g \cdot L^{-1}$).

Biochemical Analyses

At weekly intervals, a subsample of fish (n = 12) was transferred from the continuous exposure system and the gills and livers were quickly removed. The gill basket was trimmed to reduce the amount of extraneous cartilage and divided into two aliquots. Approximately half of the gill basket was immersed in 0.9% NaCl, frozen in liquid nitrogen, and stored at −60°C for assay of total ATPase and ouabain-inhibitable, Na^+-K^+-dependent ATPase. The remaining half of the gill basket and the entire liver was frozen in liquid nitrogen until analysis for metal-binding proteins.

Branchial ATPases

One half of the gill basket from each fish (52.0 ± 2.2 mg wet wt, n = 96) was homogenized on ice in 5 mL of a solution (Hendler et al. 1972) containing 0.25 *M* sucrose, 6 m*M* sodium EDTA, 20 m*M* imidazole, and 0.1% sodium deoxycholate (to make leaky vesicles). Homogenization was accomplished by 12 complete strokes of a motor-driven (500 rpm) Potter-Elvehjem grinder equipped with a teflon pestle. The cartilaginous gill arches and cell debris were removed by centrifugation for 10 min at 100 × g. The microsomal fraction was concentrated by differential centrifugation for 30 min at 10 800 × g in a Sorvall RC2B equipped with an SS-34 rotor, followed by centrifugation of the supernatant for 60 min at 100 000 × g in a Beckman L8-78 ultracentrifuge equipped with an SW 50.1 rotor. The temperature was 4°C in all centrifugations. The resulting microsomal pellet was resuspended in 5 times the tissue weight of homogenization solution minus sodium deoxycholate and frozen in liquid nitrogen until not more than 5 d later. On the day of the assay, gills from control fish (i.e. never exposed to copper, n = 6) were prepared in an identical manner and assayed to check for loss of activity and changes during storage of the microsomal suspension. No significant changes were found between freshly prepared and frozen enzyme preparations. Total ATPase and ouabain-inhibitable Na^+-K^+-ATPase activity were assayed by the NADH coupled method of Schwartz et al. (1969) as modified by (Saintsing and Towle 1978) on a Perkin-Elmer Lambda 3 spectrophotometer. The assay medium consisted of 20 m*M* imidazole (Sigma), pH 7.8, 100 m*M* NaCl, 10 m*M* KCl, 5 m*M* $MgCl_2$, 2.5 m*M* phosphoenolpyruvate, 5 m*M* Na_2ATP (Sigma), 0.5 m*M* NADH (Sigma, grade II in imidazole buffer, pH 7.4), 20 μL of pyruvate kinase − lactate dehydrogenase suspension (Sigma), and either 20 μ*M* ouabain or water for a final volume of 1.9 mL. The reaction was started, after temperature equilibration, by the addition of 10 μL of microsomal protein preparation. The change in NADH concentration was converted to inorganic phosphate (P_i) using a 1 : 1 stoichiometry. Enzyme activity was assessed at 37°C because trout Na^+-K^+-ATPase activity is greater at this temperature than at normal ambient temperature (Pfeiler and Kirschner 1972; Watson and Beamish 1980) and because mammalian enzymes were used in the ATP generating system. Protein was measured by the Coomassie Blue method of Bradford (1976).

Metal-Binding Ligands

Metal-binding ligands were assessed by two different techniques: (1) the acid-soluble thiol assay of Ellman (1959) and (2) the ^{109}Cd-binding assay of Eaton and Toal (1982).

Separate assays of acid-soluble thiols were conducted for each fish (n = 96). The other half of the whole gill basket and the entire liver were thawed and homogenized in 5 volumes of ice-cold 0.9% NaCl. At this point, 50 μL of this homogenate was removed for use in the ^{109}Cd-binding assay (see below). The remaining tissue homogenate was combined 1 : 1 with 10% TCA and the precipitated protein removed by centrifugation in a Beckman microfuge for 5 min. Total acid-soluble thiols in this supernatant were measured by the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) method of Ellman (1959) as modified by Moron et al. (1979). DTNB reacts with reduced thiols to form a compound which was measured at 412 nm on a Perkin-Elmer Lambda 3 spectrophotometer. Reduced glutathioner was the standard. Cysteine was measured by the method of Gaitonde (1967), and glutathione (both oxidized and reduced) was measured by the enzymatic method of Owens and Belcher (1965). All values were expressed as micromoles of sulfhydryl per gram of wet tissue weight. Metal-binding ligand concentration was calculated (in terms of micromoles of sulfhydryl per gram) as total acid-soluble thiol minus the sum of glutathione and cysteine (Wofford and Thomas 1984).

The 50-μL subsamples removed after homogenization in 0.9% NaCl (see above) were pooled for each sample period and combined 1 : 1 with buffer (40 m*M* Tris−HCl, pH 7.4) containing 5 m*M* dithiothreitol, a sulfhydryl reducing agent. These homogenates were heated for 10 min at 85°C and the denatured proteins removed by spinning for 5 min in a Beckman microfuge at room temperature. ^{109}Cd was added to the heat-denatured supernatant, according to the method of Eaton and Toal (1982). The heat-stable, cadmium-binding ligands in 500-μL subsamples were fractionated by Sephadex G-75 in a 2 × 30 cm column eluted at 1 $mL \cdot min^{-1}$ with 25 m*M* Tris−HCl, pH 7.4. Blue dextran, rabbit liver Cd/Zn metallothionein (types I and II, Sigma), and reduced glutathione (Sigma) were used to calibrate the column. Fractions of 1 mL each were collected and counted in a Nuclear Chicago gamma counter.

Means ± 1 standard error of the mean were calculated. Significance ($P \leq 0.05$) was tested using Student's two-tailed t-test for unpaired treatments.

Results

Copper Uptake

In control fish, whole body copper concentration (measured by atomic absorption) was 1.23 ± 0.06 $\mu g \cdot g^{-1}$ (Fig. 1a), liver copper was 23.2 ± 3.4 $\mu g \cdot g^{-1}$ (Fig. 1b), and gill copper was

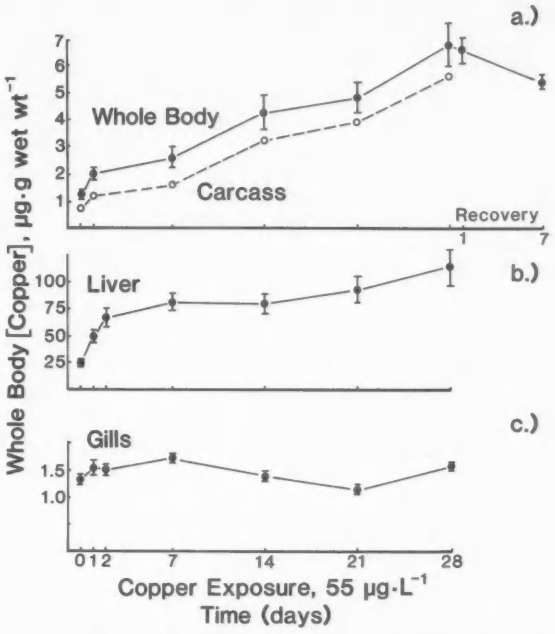

FIG. 1. Uptake (mean ± SEM) of copper in (a) whole body and carcass (n = 60), (b) liver (n = 10), and (c) gills (n = 10) of juvenile rainbow trout exposed to 55 µg copper·L^{-1} for 28 d and then transferred to clean water for 7 d.

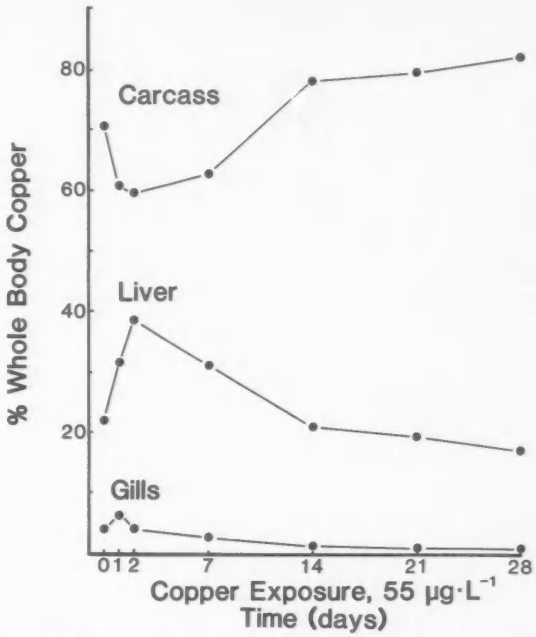

FIG. 2. Percent (mean, n = 10) of whole body copper found in carcass, liver, and gills of fish exposed to 55 µg·L^{-1} for 28 d.

1.3 ± 0.1 µg·g wet wt^{-1} (Fig. 1c). In control fish, about 24% of the whole body copper was found in the liver, with gills accounting for only 4% and the carcass (i.e. whole body minus gills and liver) accounting for the remaining 69% (Fig. 2).

During the first 48 h of copper exposure, the liver accumulated copper at a much higher rate than either gills or carcass (Fig. 1b vs. 1a and 1c) and accounted for about 40% of the whole body copper burden at 48 h (Fig. 2). However, by day 7 the accumulation rate of the liver had slowed (Fig. 1b). After 14 d, the liver accounted for only about 21% of the whole body copper burden, while the carcass now accounted for about 78% (Fig. 2). Thus, while the contribution of the liver returned to normal, that of the carcass increased. Whole body copper accumulated at a mean rate of about 33 ng·g^{-1}·h^{-1} during the first 24 h of copper exposure, but decreased to an overall mean rate of about 8 ng·g^{-1}·h^{-1} (n = 150) for the entire 28-d period; after 28 d, of exposure whole body copper reached 6.79 ± 1.8 µg·g^{-1} (Fig. 1a). Liver copper increased by about 91 ng·g^{-1}·h^{-1} during the first 48 h of exposure, and by about 74 ng·g^{-1}·h^{-1} thereafter, and reached 113.0 ± 187.0 µg·g wet wt^{-1} by day 28 (Fig. 1b). On day 28, the liver accounted for only about 17% of the whole body copper, while the carcass accounted for about 82% of the total (Fig. 2). Gill copper never accounted for more than about 6% of the whole body copper burden (Fig. 2). Gill copper increased by about 25% after 7 d of copper exposure (i.e. 1.72 ± 0.1 µg·g^{-1}), but then decreased to below control levels on day 21 (Fig. 1c). After 28 d, gill copper was 1.56 ± 0.1 µg·g wet wt^{-1}.

In the gills, the exchangeable copper pool was estimated at about 25% after 24 h of copper exposure, while the exchangeable pool of the liver was only about 4% and that of the carcass about 10%. In the gill, the size of this pool increased to 54% after 7 d of exposure and remained stable at 52.8 ± 1.3% until the end of the copper exposure. The exchangeable pool size of the liver decreased to about 2% on day 7, rose slightly on days 14 and 21, and decreased again on day 28 to 1.4%. The exchangeable pool size of the carcass also decreased gradually from about 8% on day 7 to 2.5% on day 28.

Following transfer to copper-free water, trout lost whole body copper at a rate of about 7.21 ± 0.32 ng·g^{-1}·h^{-1} (n = 10) (Fig. 1a).

Branchial ATPase Activity

The Mg^{2+}-ATPase and Na^+-K^+-ATPase specific activities in control fish were 31.6 ± 1.7 and 15.0 ± 1.8 µmol P_i·mg microsomal $protein^{-1}$·h^{-1}, respectively, at 37°C. After 24 h of exposure to 55 µg copper·L^{-1}, both of these values were reduced by about 33%. Mg^{2+}-ATPase specific activity was not significantly different from control values after 7 d of exposure, but decreased from day 7 to day 28. Na^+-K^+-ATPase specific activity remained significantly depressed and relatively constant from 24 h to 28 d of exposure (Fig. 3). After 24 h in clean water, both Mg^{2+}-ATPase and Na^+-K^+-ATPase specific activities increased, but the change was not significant. However, after 7 d of recovery, there was no significant difference in the specific activity of either enzyme from control values (Fig. 3).

Microsomal protein was also measured as a function of whole gill wet weight (Fig. 3). After 24 h of copper exposure, the amount of microsomal protein per milligram of gill tissue increased, and this trend became significant after 7 d and continued for the 28 d of exposure (Fig. 3). After 24 h of recovery in clean water, the microsomal protein concentration again increased and was significantly higher than control values by day 7 of recovery (Fig. 3).

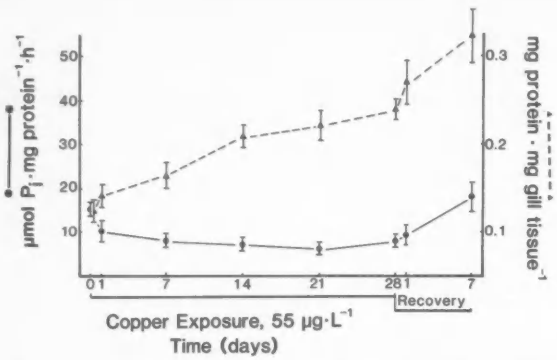

FIG. 3. Branchial, microsomal Na^+-K^+-ATPase specific activity (●) and total microsomal protein (▲) (mean ± SEM, n = 12) in juvenile rainbow trout exposed to 55 μg copper·L^{-1} for 28 d and then transferred to clean water for 7 d.

The mean total gill Na^+-K^+-ATPase activity (i.e. Na^+-K^+-ATPase specific activity times total microsomal protein per milligram gill wet weight) was 2009 ± 382 μmol P_i·h^{-1} under control conditions. After 24 h of copper exposure, this total gill activity was reduced by about 33% to about 1363 ± 313 μmol P_i·h^{-1}, but had recovered to 2166 ± 448 μmol P_i·h^{-1} by day 14. After 7 d of recovery, the total gill activity was about 4815 ± 1118 μmol P_i·h^{-1}, or 2.5 times that found under control conditions.

Gill and Liver Acid-Soluble Thiols

In control fish, acid-soluble thiol (AST) concentrations were 0.98 ± 0.06 μmol sulfhydryl·g wet wt^{-1} in gills and 2.28 ± 0.12 μmol·g^{-1} in liver (n = 12) (Fig. 4). Glutathione accounted for nearly all of this total thiol, with concentrations of 0.91 ± 0.04 and 1.87 ± 0.13 μmol·g^{-1} for gill and liver, respectively. Gill cysteine was 0.08 ± 0.004 μmol·g^{-1} and liver cysteine was 0.07 ± 0.004 μmol·g wet wt^{-1} and therefore did not make a significant contribution to the total AST. Glutathione accounted for about 85% of liver AST and greater than 98% of gill AST under control conditions.

Gill AST and glutathione did not change significantly during the entire 28-d period of copper exposure (Fig. 4). However, by day 7, liver AST had about doubled and the non-glutathione, non-cysteine component of hepatic AST had increased to about 2.8 times above that found under control conditions (Fig. 4). Liver glutathione and cysteine accounted for about 73% of the total AST on day 28.

After 7 d of recovery in copper-free water, gill AST and glutathione were unchanged (Fig. 4), but large decreases in both AST and glutathione were found in the liver. In the liver, however, the non-glutathione, non-cysteine component of AST did not decrease significantly.

Metal-Binding Ligands in the Gill and Liver

Standard Cd/Zn metallothionein eluted from the Sephadex G-75 column between fractions 25 and 35, with a peak at fraction 31. Therefore, we have arbitrarily designated fractions 1−24 as high molecular weight (HMW), fractions 25−35 as medium molecular weight (MMW), and fractions 36−60 as low molecular weight (LMW). We found a distinct protein peak in trout liver, which has the same approximate molecular

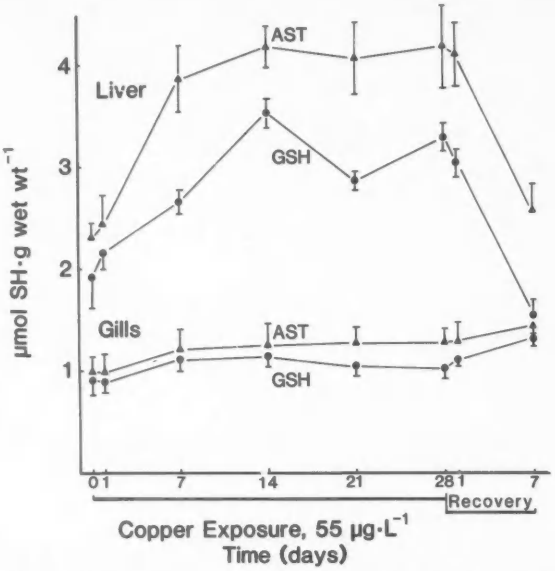

FIG. 4. Branchial and hepatic acid-soluble thiols (AST) and glutathione (GSH) (mean ± SEM, n = 12) in juvenile rainbow trout exposed to 55 μg copper·L^{-1} for 28 d and then transferred to clean water for 7 d.

weight as our Cd/Zn metallothionein standard. This protein is also heat-stable and has a high affinity for cadmium.

In control fish, most of the ^{109}Cd bound to gill supernatants was found in the HMW fractions, and no peak was found in the region where standard metallothionein eluted; this continued for the duration of the copper exposure and recovery period (Fig. 5). On the other hand, in control liver, about 80% of the ^{109}Cd binding was associated with proteins of about 10 000 daltons, with relatively little binding to HMW and LMW proteins (Fig. 5). There was no significant change in ^{109}Cd binding in liver after 24 h, but ^{109}Cd binding decreased progressively from day 7 to 28, so that at day 28, ^{109}Cd binding was only about 50% that of control livers (Fig. 5). No change was found after 7 d of recovery in copper-free water (Fig. 5).

Discussion

Uptake and Distribution of Copper

The liver of mammals acts as the major organ for copper storage and is the first line of defense against copper poisoning (Buck 1978). Copper becomes toxic to mammals only when the normally high binding capacity of the liver is exceeded and copper is released into the bloodstream where it causes hemolysis, icterus, and anemia. In fish, the liver is also the major storage organ for copper (Buckley et al. 1982; Shearer 1984), but the presence of gills makes for a critically important difference between fish and mammals. In previous studies we have shown that the gills of trout are very sensitive to copper (Laurén and McDonald 1985), and in the present study we have shown that they also have a very low copper-binding capacity. In fish, therefore, hepatic copper accumulation can only become important to survival if the gills are able to withstand elevated copper concentrations (Laurén and McDonald 1985). Furthermore, the fish liver cannot be treated as the sole site of internal

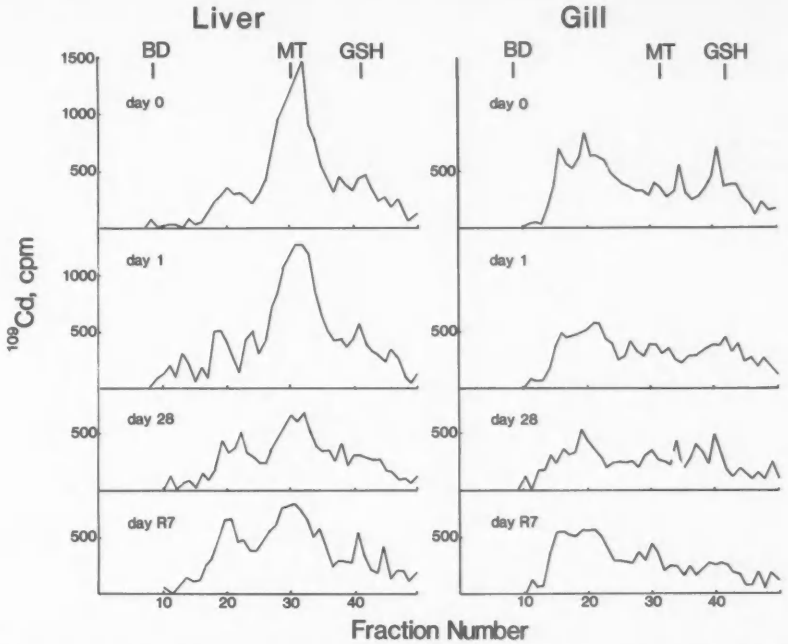

FIG. 5. Sephadex G-75 separation of in vitro hepatic and branchial ^{109}Cd binding in pooled, heat-denatured supernatants from juvenile rainbow trout exposed to 55 μg copper·L^{-1} for 28 d and then transferred to clean water for 7 d. BD, blue dextran; MT, Cd/Zn metallothionein; GSH, glutathione.

copper accumulation. In the present study, the copper-binding capacity of the liver reached an apparent plateau after about 7 d, and further copper uptake was distributed to other internal tissues. Since white muscle makes up about 66% of the whole body wet weight of rainbow trout (Stevens 1968), this is the most likely site for extra hepatic copper accumulation. However, it must be cautioned that the carcass, as defined in the present study, included, in addition to muscle, the kidney, spleen, gut, and brain, and although copper uptake into these tissues is very low relative to either the liver or gill (D. J. Laurén, unpublished), little is known about the physiological sensitivity of these tissues to copper. This is clearly an important area for future research.

Whole body copper continued to increase, and did not reach equilibrium during the 28 d of exposure. Interestingly, Dixon and Sprague (1981a) found that fish exposed to 30, 131, and 194 μg·L^{-1} reached apparent equilibrium, but fish exposed to intermediate levels, 58 and 94 μg·L^{-1} were less tolerant, while those exposed to the higher levels were more tolerant during subsequent lethality trials. Thus, the relationship between copper uptake, equilibrium, and resistance is not clear at the present time.

The loss of whole body copper at approximately the same rate that copper was accumulated suggests that despite the high affinity of copper for biological ligands (Nieboer and Richardson 1980), and the apparent incorporation of copper into nonexchangeable or slowly exchangeable pools, the whole body copper pool is quite labile once copper exposure has ceased. We have previously shown that a large portion of the apparent copper uptake during short-term experiments is due to surface binding (Laurén and McDonald 1987), and this may explain a portion of the loss of whole body copper following transfer to clean water, but the magnitude of the losses found in the present study suggests that internalized copper was the predominant source.

Response of the Gills

The inhibition and induction of Na^+-K^+-ATPase is the most likely explanation for the inhibition and recovery of sodium uptake reported in the previous paper (Laurén and McDonald 1987). However, while similar trends were found between the in vitro and in vivo measurements, perfect correlation was not found. For example, the specific activity of branchial Na^+-K^+-ATPase was inhibited by about 33%, but sodium uptake was inhibited by about 54% (Laurén and McDonald 1987). Furthermore, the total branchial transport capacity (i.e. specific activity times total microsomal protein) returned to control values by day 14, but sodium uptake did not recover completely until day 21 (Laurén and McDonald 1987). This emphasizes the importance of conducting in vivo studies whenever possible and suggests, as might be expected, that in vitro assays do not necessarily reflect in vivo conditions and/or that copper has effects on other components of the sodium uptake mechanism (i.e. sodium channels, mitochondria, membrane fluidity, etc.).

Copper was not significantly elevated in the gills even at the point of greatest inhibition of sodium uptake and Na^+-K^+-ATPase inhibition. This may indicate either a rapid turnover of copper binding ligands within the gill or that very little copper may be needed to inhibit this enzyme. We are not aware of any data concerning the turnover rates of gill enzymes, but Reidel and Christensen (1979) reported that copper inhibits Na^+-K^+-ATPase in vitro at about a 1:1 molar ratio. Under control conditions, we measured about 130 μg microsomal protein·mg

gill tissue^{-1}. For our average gill weight of about 104 mg, this is 13.5 mg of microsomal protein. At an estimated molecular weight of 250 000 $\mu g \cdot \mu mol^{-1}$ for Na^+-K^+-ATPase (using Coomassie Blue; Stekhoven and Bonting 1981), and assuming that all of the microsomal protein was Na^+-K^+-ATPase, this is only about 54 nmol of enzyme; such a small change in tissue copper concentration copper would have been below our limits of detection. Furthermore, copper does not have to be irreversibly bound to inhibit enzymes and can enter into oxidation–reduction cycles with sulfhydryl groups to form disulfide bonds (Rothstein 1959) and/or can catalyse the formation of cytotoxic free radicals from membrane phospholipids (Hochstein et al. 1980; Florence and Stauber 1986).

Neither AST (Fig. 4), glutathione, cysteine, or ^{109}Cd-binding proteins (Fig. 5) were induced by copper exposure. Furthermore, if copper were tightly bound by the gill, one would expect the exchangeable copper pool to decrease, rather than increase or remain stable, as was found in the present experiment. Apparently, then, the induction of metal-binding ligands has no role in the recovery of sodium uptake, at least at 55 μg copper$\cdot L^{-1}$. Nöel-Lambot et al. (1978) found that the gills of eels (*Anguilla anguilla*) also have a very low capacity to induce metal-binding ligands.

Response of the Liver

Copper exposure led to copper accumulation by the liver and the apparent induction of AST and glutathione synthesis. Wofford and Thomas (1984) have shown that in the striped mullet (*Mugil cephalus*), AST includes oxidized and reduced glutathione, cysteine, and an acid-soluble, sulfhydryl-rich, cadmium-inducible, metal-binding protein of about the same molecular weight as metallothionein. We have also shown that the sum of glutathione and cysteine does not equal the total AST and that an acid-soluble, sulfhydryl-rich, cadmium-binding protein of about 10 000 daltons is present in the livers of control trout. On these criteria, we suggest that this protein is also metallothionein. Furthermore, this protein was induced in the liver of trout within 7 d of exposure to copper, but then remained at a relatively constant, high level. During the same period, the size of the exchangeable copper pool decreased and then remained at a constant low level. Such results might be expected if metallothionein was induced and copper bound more firmly and suggest that measurements of exchangeable pool size may be a useful method for estimating changes in metal-binding capacity in vivo.

On the other hand, ^{109}Cd binding in the liver decreased as copper exposure continued. This occurred because metallothionein binds metals in the sequence copper > cadmium $\gg$ zinc (Rupp and Weser 1978; Furey et al. 1986.) All naturally occurring metallothionein contains zinc, and both copper and cadmium are incorporated into metallothionein by displacing zinc (Cousins 1985; Day et al. 1984; Nöel-Lambot et al. 1978). However, cadmium cannot displace copper from metallothionein (Nöel-Lambot et al. 1978; Scheuhammer and Cherian 1986). Therefore, during copper exposure when the ratio of copper to zinc in metallothionein increases, the cadmium-binding capacity of metallothionein decreases. It is apparent then that hepatic detoxification of copper may act in two distinct ways: (1) by the induction of metallothionein and (2) by increasing the percentage of metal binding sites occupied by copper. In this respect it is important to note that Goering and Klassen (1984) reported that the increase in lethal resistance found when rats are pretreated with cadmium is dependent on presynthesized metallothionein, rather than an increase in the rate of metallothionein production. Thus, measurement of metallothionein by such indirect assays as ^{109}Cd binding, pulse polarography, or radioimmunoassay may seriously misrepresent the true detoxifying potential of a given tissue.

The induction of hepatic metallothionein has been considered the most satisfactory explanation of the acquired resistance of animals to metal toxicity (Dixon and Sprague 1981b; Brown and Parsons 1978; Webb and Cain 1982; Cousins 1985). However, there is no evidence for copper-induced liver dysfunction in fish exposed to environmentally relevant levels of copper (Baker 1969; Gardner and LaRoche 1973), and hepatic metallothionein may be induced by a variety of nonspecific stresses, as well as the glucocorticoid hormones (Cousins 1985). Furthermore, McCarter and Roch (1983) showed that although hepatic metallothionein was induced by copper exposure in coho salmon (*Oncorhynchus kisutch*), the increase in resistance actually preceded the increase in metallothionein. Thus, at least for copper, the increase in hepatic metallothionein may be a secondary, rather than a primary, response. On the other hand, our studies have shown that the gill is the primary target of copper toxicity in rainbow trout (Laurén and McDonald 1985, 1986, 1987), and there is clear evidence that zinc, mercury, and cadmium act at the gills as well as internal organs of fish (Spry and Wood 1984, 1985; Renfro et al. 1974; Bouquegneau 1977). Therefore, to the extent that these metals affect ionoregulatory function, the classical mammalian model for the hepatic detoxification of metals may not hold true.

Acknowledgements

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Distribution and Persistence of the Lampricide Bayer 73 Following a Control Application

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Ho, K. T. Y., and S. P. Gloss. 1987. Distribution and persistence of the lampricide Bayer 73 following a control application. Can. J. Fish. Aquat. Sci. 43: 112–119.

Bayer 73 (2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide) was applied to a 41-ha delta area of Seneca Lake, New York, to control the sea lamprey (*Petromyzon marinus*). Concentrations of Bayer 73 in lake water samples ranged from <10 to 573 μg/L (ppb); concentrations peaked 2.5 h after application and were generally highest near the bottom and lowest at the surface, but exceeded 40 μg/L throughout the water column. All measured concentrations were 10 μg/L (limit of detection) or less within 96 h after application. Movement of the chemical out of the treatment area, as well as drift within the treatment area, was detected. Significant mortality of one species of caged fish occurred at the station with the highest Bayer 73 concentration 14 h after application. Fish tissue residues (<10–858 ng/g) reflected water samples in concentration and distribution patterns; they were highest 14–24 h after application and then declined. In laboratory studies performed to determine the mechanisms affecting the decline in Bayer 73 concentrations, sediment processes — specifically adsorption — enhanced the decline.

Du Bayer 73 (sel 2-aminoéthanol de 2′,5-dichloro-4′-nitrosalicylanilide) a été répandu sur 41 ha dans un secteur deltaïque du lac Seneca, New York, afin de lutter contre la lamproie de mer (*Petromyzon marinus*). Dans les échantillons d'eau du lac, la concentration de Bayer 73 variait entre <10 à 573 μg/L; la concentration était à un maximum au bout de 2,5 h après l'épandage; elle était généralement plus élevée au fond qu'en surface du lac, mais elle dépassait 40 μg/L partout dans la colonne d'eau. Toutes les mesures de concentration étaient égales ou inférieures à 10 μg/L (limite de détection) en moins de 96 h après l'épandage. Une migration de l'agent chimique hors de la zone traitée, ainsi qu'une dérive à l'intérieur, ont été détectées. Quatorze heures après l'épandage, il y a eu une mortalité importante chez une espèce de poisson tenus en cage à la station où était enregistrée la plus forte concentration de Bayer 73. La concentration des résidus dans les tissus de poissons (<10–858 ng/g) suivait assez bien la concentration dans les échantillons d'eau et la distribution; elle était le plus élevé entre 14 et 24 h après l'épandage. Dans les tests en laboratoire visant à déterminer les mécanismes qui interviennent dans la diminution de la concentration du Bayer 73, il a été noté que des processus sédimentaires, notamment l'adsorption, accentuaient cette diminution.

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Bayer 73 or Bayluscide (here termed Bayer) are trade names for the ethanolamine salt of 2′,5-dichloro-4′-nitrosalicylanilide. Bayer is registered in the United States for use (1) in surveys to assess the density of larval sea lamprey (*Petromyzon marinus*) and (2) as an additive (up to 2%) with 3-trifluoromethyl-4-nitrophenol (TFM) to control larval sea lamprey in streams (Meyer and Schnick 1983).

Bayer alone reduces ammocoete densities in field applications (Manion 1969); however, data on its dissipation or dispersion in a water mass are limited to a study by Newton and Fetterolf (1967) when the limit of detection was 300 μg/L — well above the toxic level for many aquatic organisms (Marking and Hogan 1967).

The application of Bayer to Seneca Lake, New York, afforded us the opportunity to monitor the environmental fate of the chemical. The purposes of our field study were to describe the distribution, dispersion, and dissipation of Bayer in the water column after an application and to assess its bioaccumulation by, and toxicity to, two species of caged, non-target fish. The effectiveness of this control treatment in reducing sea lamprey ammocoetes was described by Engstrom-Heg (1983). Two laboratory experiments were performed to examine processes that may be important in the disappearance of Bayer under simulated environmental conditions. The objectives of the laboratory experiments were to determine (1) which compartment — water or sediment — controlled the disappearance of Bayer and (2) the relative rate of photolysis and hydrolysis in the water column and (3) to examine the effects of microbial degradation.

Materials and Methods

As part of a sea lamprey control program, the New York State Department of Environmental Conservation applied 5 t of Bayer to Seneca Lake, one of the Finger Lakes of western New York (Fig. 1), on September 9, 1982. The 41-ha treatment area was a shallow delta with a maximum depth of 4.5 m and without thermal stratification. Bayer was manufactured by Mobay Chemical Company and applied aerially in a 5% gran-

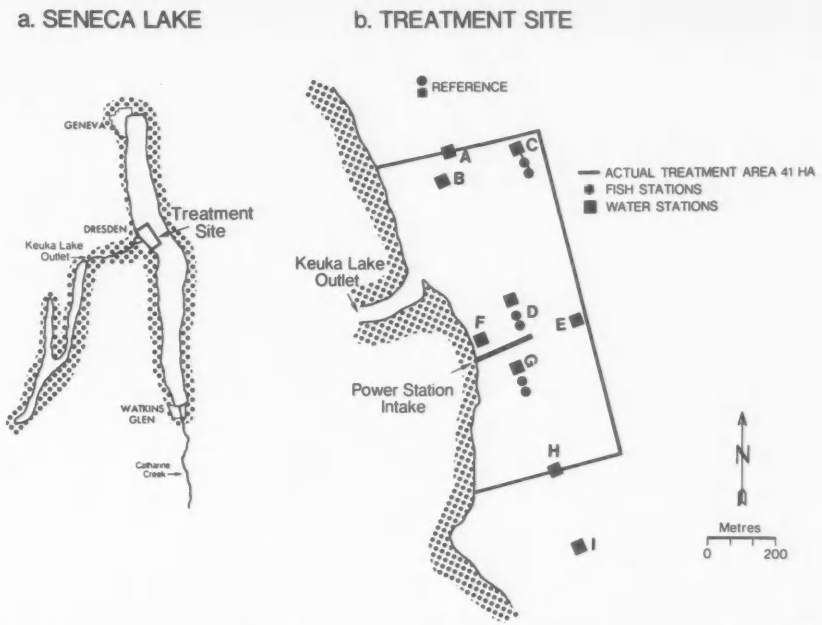

FIG. 1. Location of (a) treatment areas in Seneca Lake and (b) caged fish and water sampling stations (A–I) at the site.

lar formulation at a nominal rate of 110 kg/ha, or 2.3 mg/L (assuming dissolution into the 10-cm layer of water immediately above the lake bottom).

Water Samples

Nine water sampling stations were established at Dresden, New York (Fig. 1). Each station was sampled at depths of 0.1, 0.25, 0.5, and 1 m above the substrate, as well as at middepth and the surface. Station depths (metres) were as follows: A, 4.5; B, 3.2; C, 4.5; D, 2.5; E, 3; F, 1; G, 2.6; H, 2.5; and I, 2.5.

Pneumatically operated samplers (Blakar 1979) were used to sample discrete depth intervals in the water column and close to the substrate. Samples were emptied into 10-mL Teflon-lined screw-cap glass test tubes, frozen on dry ice at the site, and kept frozen until 12 h before analysis. Water stations were sampled every 30 min through the first 3 h postapplication every 60 min through the next 3 h, and once every 6 h through 24 h. Samples were then collected daily through 96 h.

Fish Samples

Four stations were established to determine mortality of nontarget fish and the accumulation of Bayer residue in their muscle tissue. Three stations were in the treatment area and one was located outside it (Fig. 1).

Largemouth bass (*Micropterus salmoides*) and rainbow trout (*Salmo gairdneri*) were placed at each station in paired wire cages (20 × 70 × 70 cm) at 0, 1, and 2 m above the substrate 10–18 h before Bayer was applied; 35 each of rainbow trout (mean total length 157 cm, mean weight 42 g) and largemouth bass (182 cm, 91 g) were placed in three separate cages at each site. The fish were observed for mortality immediately before the application and sampled at 8, 14, 24, 48, 96, and 168 h posttreatment. Cages were brought to the surface at each sampling period by divers using scuba, and three live and all dead fish were removed. The live fish were narcotized with benzocaine, wrapped in aluminum foil, and frozen with dry ice on site.

Laboratory Experiments

Two sequential experiments were performed to identify the most probable mechanism for loss of Bayer from the water column. Sediment and water samples were collected from the treatment site on October 3, 1983, for experiment I and on November 4, 1983, for experiment II.

In experiment I, designed to test for the effect of sediment processes, photolysis, and hydrolysis on Bayer loss, 3 L of Bayer at 500 μg/L (ppb) in lake water was added to each of eight beakers. Five centimetres of sediment was added to two of the beakers (sediment processes, photolysis, hydrolysis). Two of the beakers without sediment were wrapped with aluminum foil to exclude light (hydrolysis). Four beakers were not wrapped and contained no sediment (photolysis). All beakers were covered with Pyrex lids and randomly placed in a water bath (21°C) in natural sunlight. Water samples were taken 10 cm above the substrate (sediment or beaker bottom) every 30 min for the first 3 h, every 1 h for 6 h, every 6 h for 3 d, and once every 12 h for 14 d.

We were interested in natural light condition in experiment I; however, we were unable to partition microbial degradation under these conditions. In the second experiment, four treatments were applied. In two, sterilized sediment and Bayer solution (one light-adsorption, hydrolysis, photolysis and one dark-adsorption, hydrolysis) were used; in the third, only sterilized Bayer solution was used (hydrolysis), in darkness; and in the fourth, unsterilized sediment and Bayer solution were ex-

posed to light (adsorption, hydrolysis, photolysis, and microbial degradation). Sterilized Bayer solution (300 μg/L) was prepared by autoclaving lake water filtered through 0.22-μm Millex GS filters before they were placed in 10-mL Teflon-lined screw-cap pyrex test tubes. Sediment was sterilized with ^{60}Co irradiation. All sediment manipulations were performed under a sterile laminar flow hood. "Dark" test tubes were wrapped in aluminum foil. Test tubes were placed (without shaking) into an incubator at 21°C under constant light (1300 lm). After a 12-h equilibration, two test tubes were taken from each treatment every 24 h for 2 wk. Aliquots from each test tube were plated on nutrient agar and observed over 2 wk to test for sterility.

Sediment and water analyses were performed by the Cornell University Agronomy Analytical Testing Laboratory. Organic and total carbon in sediment were determined by wet combustion (Allison 1960). Organic carbon in water was determined by oxidation with $K_2Cr_2O_7$ and back-titration of excess $Cr_2O_7^{2-}$ with $FeSO_4$, a modification of a method for soils (Walkley 1947). Sediment contained 0.34% organic carbon and 0.77% total carbon; and water analysis showed 3.2 μg organic carbon/mL. Data on incident sunlight were obtained from the Cornell University Meteorology Unit. Middle and long-wave UV values equaled 2% of total solar radiation (Miller and Thompson 1979).

Linear regressions were performed on log concentration versus time data for experiment I beakers with sediment in light compared with beakers without sediment in light. Beakers wrapped in aluminum foil and two of the beakers without sediment were regressed on data for log concentration versus langley (1 langley = 4.1855 absolute J/cm^2). Comparison of these beakers with sediment beakers was based on langley equivalents of nine full sunny days in the Ithaca (New York) area during October (382 langleys per sunny day is an empirical value). Students *t*-test, to determine differences between treatment slopes, and linear regressions, on log concentration versus time or equivalent langley data, were performed by using minitab (Ryan et al. 1976) in experiment I. We determined differences between treatments in experiment II by contrasts and multiple linear regression, using Statistical Analysis Systems (Helwig and Council 1979). All significant values are at $P \leq 0.05$.

To analyse water samples in the laboratory, we used a modification of the method described by Luhning et al. (1979). Two millilitres of each water sample was pipetted into a glass test tube, made 2 *M* by the addition of 2 mL of 4 *M* NaOH. The samples were then hydrolyzed in a water bath (100°C) to yield 2-chloro,4-nitroaniline (CNA). The CNA was extracted into 2 mL of hexane–ether (75:25 v/v) and analyzed by gas–liquid chromatography with electron capture detection. The lower detection limit was 10 μg/L; the standard error was 1.4% at 20 μg/L and 2.8% at 100 μg/L. Standards preserved by freezing for over 6 mo produced recoveries of 97% ($N = 5$) and 101% ($N = 2$) at the 100- and 20-μg/L level, respectively. Hence, values are reported uncontrolled for aging or recovery.

Fish were filleted according to standard New York State Department of Environmental Conservation procedures (L. Skinner, New York State Department of Environmental Conservation, 50 Wolf Road, Albany, NY, pers. comm.). Muscle fillets from each sample of three fish were combined to form a single sample. In analyzing fish samples, we followed the method of Luhning et al. (1979). Spiked samples of rainbow trout fillets produced mean recoveries of 108% ($N = 3$) and 100% ($N = 4$) at levels of 100 and 135 ng Bayer/g, respectively. The level of detection for Bayer in fish samples was 45 ng/g, although absolute levels less than 80 ng/g should be considered tentative because of some daily variation in performance of the gas chromatograph.

Results and Discussion

Environmental Monitoring

Lake water

Bayer in lake water samples ranged from <10 to 573 μg/L. The highest concentration was at station G, depth 0.1 m, 2.5 h after application (Table 1). Concentrations were generally lowest at the surface and highest at the bottom (0.1 m), but concentrations greater than 40 μg/L were measured at all depths and stations within the treatment area. All concentrations were below 30 μg/L 48 h after application and were at or below the detection limit of 10 μg/L 96 h after application.

At the deeper stations (B, C, D, and G), (1) concentration increased with depth, (2) concentration increased until 2.5 h after application, (3) surface concentrations increased at 6–18 h, and (4) concentration declined 3–4 h after application in the northern part of the treatment area (stations B, C, and D) and 6 h after application in the southern part of the treatment area (station G). At station F the relatively high and uniform concentrations of 200–300 μg/L were probably due to its shallow depth (1 m) and mixing.

The spatial and temporal dispersion of Bayer in Seneca Lake was apparently affected by both horizontal and vertical movement and mixing of the water mass. Boundary stations A, E, H, and I showed different patterns, depending on their location. Concentrations were low (<10–60 μg/L) at station A, on the northern edge of the treatment area, until 5 h after application, when concentrations increased to 321 μg/L and then declined to less than 10 μg/L by 72 h after application. At station E on the eastern edge of the treatment area, concentrations were low (<40 μg/L) until 12 h after application, when surface concentrations increased to 87 μg/L before declining to less than 10 μg/L by 48 h. The much lower levels of Bayer at boundary stations than in the treatment area immediately after the application indicated that the application was restricted to the prescribed area. However, later movement of the chemical within the water mass was apparent. North to south movement of the chemical was observed at station H on the southern edge and at station I, 100 m south of the treatment area. This southward movement of the chemical out of the treatment area (Table 1) was attributed to normally prevailing north to south currents at the treatment site. Most concentrations were less than 10 μg/L at station H until 12 h after application, when they increased to an average of 284 μg/L and then declined to less than 10 μg/L by 96 h. At station I, the same pattern was observed, except that peak levels were observed at 18 h instead of 12 h and the average concentration was 83 μg/L. Some northward movement of the chemical near the boundary of the treatment area, evidenced by low Bayer residues in caged fish north of the treatment area and increased levels in water from stations A and B at 6 h, could be attributed to simple diffusion or movement of the chemical in the water mass.

Disappearance of the chemical over time may be attributed to either movement, dissipation, or degradation. Declines to less than 30 μg/L within 48 h and to less than 10 μg/L within 96 h corresponded well with rates reported in the literature.

TABLE 1. Concentrations (μg/L) of Bayer 73 levels in lake water samples collected from treatment areas in Seneca Lake following a control application (numerical values are distances above substrate in metres, S = water surface, M = middepth).

		Time posttreatment (h)														
Station[a]	Depth	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	6.0	12.0	18.0	24.0	48.0	72.0	96.0
A	S	63	17	22	43	29	53	51	123	46	46	18	18	0[b]	0	0
	M	0	0	19	16	18	19	11	30	31	0	18	10	0	0	0
	1.0	0	0	14	17	26	31	30	49	51	10	0	9	0	0	0
	0.5	0	0	13	29	21	31	38	51	103	10	0	13	0	0	0
	0.25	0	0	13	30	23	47	33	283	108	10	0	15	0	0	0
	0.1	0	0	13	23	33	60	52	321	144	0	0	15	0	0	0
B	S	44	31	26	39	27	56	113	123	93	59	26	24	0	0	0
	M	28	31	20	37	22	30	31	35	32	10	26	39	0	0	0
	1.0	40	34	39	50	38	44	26	27	96	10	0	38	0	0	0
	0.5	23	36	62	80	106	61	47	75	152	0	0	38	0	0	0
	0.25	—	35	40	114	193	72	64	132	133	13	13	40	0	0	0
	0.1	23	48	107	175	131	117	115	141	148	16	13	45	0	0	0
C	S	16	26	27	11	19	40	22	102	132	36	24	13	11	0	0
	M	16	14	27	0	0	0	0	17	0	0	0	0	0	0	0
	1.0	22	16	13	7	12	7	0	0	0	0	0	0	0	0	0
	0.5	27	23	27	11	13	8	8	0	0	0	0	0	0	0	0
	0.25	42	41	28	10	18	10	16	9	14	0	0	0	0	0	0
	0.1	46	46	50	—	23	18	20	11	14	0	0	0	0	0	0
D	S	28	20	18	13	22	19	39	26	30	49	32	57	14	0	0
	M	17	13	17	11	18	13	27	15	70	108	0	0	—	—	0
	1.0	28	30	16	9	18	11	79	152	78	65	0	0	11	0	0
	0.5	33	137	16	65	112	58	62	148	137	60	0	0	11	0	10
	0.25	52	139	135	97	130	50	92	147	131	60	14	0	16	0	10
	0.1	118	168	176	92	138	—	109	147	127	54	14	0	16	10	10
E	S	0	—	25	11	0	0	0	0	0	30	24	87	12	0	0
	M	0	28	34	13	0	0	0	0	0	0	0	0	0	0	0
	1.0	18	19	18	0	11	0	0	0	0	0	0	0	0	0	0
	0.5	16	16	15	0	0	0	0	0	0	0	0	0	0	0	0
	0.25	17	0	13	0	0	0	0	0	0	0	0	0	0	0	0
	0.1	16	0	15	0	0	0	0	0	0	0	0	0	0	0	0
F	1.0	32	107	303	256	341	334	416	420	345	309	103	74	26	9	0
	0.5	23	59	146	206	276	320	266	279	204	131	112	66	26	9	0
	0.25	32	131	235	207	279	256	269	265	200	150	130	108	0	9	0
	0.1	72	248	267	228	293	262	244	275	219	149	133	112	24	9	0
G	S	0	0	0	0	0	0	0	0	0	109	31	25	13	0	0
	1.0	0	0	12	14	36	10	12	0	0	70	83	32	12	0	0
	0.5	20	38	39	60	131	164	11	0	24	207	72	52	15	8	0
	0.25	30	52	89	69	460	182	14	0	99	250	82	49	18	0	0
	0.1	39	35	461	556	573	228	23	0	21	252	81	47	20	14	0
H	S	0	0	0	0	0	0	0	0	0	155		25	12	0	—
	1.0	0	0	0	0	0	0	0	0	0	157	202	44	15	0	0
	0.5	0	0	0	0	0	0	0	0	0	359	155	124	13	8	0
	0.25	21	13	0	0	0	0	0	0	0	363		98	18	8	0
	0.1	55	97	0	42	0	10	0	0	0	386	115	99	20	14	0
I	0.0	0	0	0	0	0	0	0	0	0		22	15	0	0	0
	1.0	0	0	0	0	0	0	0	0	0	0		33	16	0	0
	0.5	0	0	25	0	0	0	0	0	0	0	116	80	19	0	0
	0.25	0	0	0	0	0	0	0	0	0	13	93	99	20	0	0
	0.1	0	0	0	0	0	0	0	0	0	13	102	107	18	0	0

[a]See Fig. 1 for location of stations.
[b]<10 μg/L (detection limit in water).

Webbe (1961) reported that detectable concentrations (trace detection at 200 μg/L) of the chemical had disappeared within 48 h in experimental ponds. Strufe et al. (1965) observed a 50% decrease of 1000 μg Bayer-L applied to a slow-flowing canal 25 h after application.

Newton and Fetterolf (1967) reported no detectable Bayer 15 cm above the bottom in Houghton Lake, Michigan, at 2, 5, and 22 h after an application; however, their limit of detection

TABLE 2. Concentrations of Bayer 73 (ppb wet wt) in standard fillet samples from caged fish after control application in Seneca Lake (—, no sample taken; <45 indicates trace amount below quantifiable limit).

Station[a]	Depth (metres from bottom)	Largemouth bass time posttreatment (h)						Rainbow trout time posttreatment (h)					
		8	14	24	48	96	168	8	14	24	48	96	168
Control	0	<45	0	0	<45	0	0	<45	<45	<45	0	0	75
	1	<45	<45	<45	<45	—	55	0	0	0	0	0	<45
	2	0	<45	61	0	—	0	<45	<45	<45	<45	0	0
C	0	52	—	83	0	0	70	46	0	<45	0	<45	0
	1	0	75	62	0	0	0	0	0	0	0	<45	<45
	2	<45	94	51	0	0	0	0	0	0	0	0	0
D	0	288	151	470	363	106	142	208	217	379	128	<45	0
	1	0	356	124	155	162	0	—	268	81	0	0	—
	2	109	238	264	181	0	—	116	513	144	89	63	0
G	0	—	367	858	391	780	—	—	338	—	—	—	—
	1	0	—	322	325	—	118	0	266	301	72	80	—
	2	264	<45	71	73	0	0	0	0	85	0	0	—

[a]See Fig. 1 for location of stations.

was 300 μg/L (trace detection at 100 μg/L). Relatively high surface concentrations at 6–18 h after application in Seneca Lake could have been due to wind-induced surface movement of the chemical, drift from prevailing currents, or dust from the application.

Bayer was applied at a nominal rate of 110 kg/ha, or an expected concentration of 2300 μg/L (assuming dissolution into the bottom 10 cm of water). Expected concentrations were not achieved because dissolution occurred throughout the water column rather than in only the bottom 10 cm. However, when concentrations were weighted by water volume to estimate an application rate (in weighting, we used an area of 1 m^2 at the time of highest concentration at each station), the nominal and observed rates were similar. There was a large difference between the expected concentration of 2300 μg/L and the observed concentrations of 23–573 μg/L. Weighting the observed concentration by volume yielded a range of 113–295 mg/m^2, compared with an expected rate of 230 mg/m^2.

Weighting concentration by volume at each station also decreased the amount of variation among stations. Concentrations ranged from 23 μg/L at station C to 573 μg/L at station G, 2.5 h after application — a 25-fold difference. However, average Bayer concentration weighted by volume at 2.5 h after application ranged from 43 mg/m^3 at station C to 295 mg/m^3 at station F — less than a sevenfold difference.

Because the analytical procedure used (Luhning et al. 1979) enabled detection of CNA and not Bayer, we were concerned about observing CNA rather than Bayer in both the field and laboratory studies. However, Schultz and Harman (1978) reported no degradation of ^{14}C-labeled Bayer within 56 d in either buffered aqueous solution or pond water, and El-Dib and Aly (1976a) showed that chemical hydrolysis (at neutral pH < 9) was a minor route of Bayer degradation. Treatment area pH was 7–8. Water temperature ranged from 19 to 25°C and pH from 7.02 to 7.65, bottom to surface, respectively, at station C.

Fish

Bayer residues in fish muscle tissue reflected water concentration and distribution patterns. Residues ranged from 0 to 858 ng/g and were highest in fish from the bottom depth at all stations (Table 2). Residues increased until 14–24 h after application and then declined. Bayer residues were highest in largemouth bass at the bottom depth of station G and lowest in rainbow trout at station C. Concentrations of Bayer were higher in largemouth bass than in rainbow trout at all stations.

The only substantial mortality of caged fish (90%) was observed at 14 h after application in the rainbow trout cage on the substrate at station G. No largemouth bass were dead at the station. The difference in mortality of rainbow trout (90%) and largemouth bass (nil) at station G 14 h after application was probably due to the difference in the LC_{50}'s for these species. Marking and Hogan (1967) reported 24-h LC_{50}'s of 52 μg/L (95% confidence interval 49–56 μg/L, slope = 1.13) for rainbow trout and 111 μg/L (95% confidence interval 99–124 μg/L, slope = 1.13) for largemouth bass. The narrow confidence interval and steep slope function supported the difference in mortality observed between species at the same station. Excluding this incident, the average fish mortality for the first 48 h was zero for largemouth bass and 1.3% for rainbow trout. Additional mortality during the posttreatment period was attributed to cage effects and fungal infections, but treatment mortality was no different or less than in reference cages (Ho 1985).

The selective toxicity of the granular formulation of Bayer has been based on dissolution at the mud–water interface. Because of this localized distribution, it has been assumed that nontarget fish could escape lethal concentrations of Bayer whereas ammocoetes, which live in the substrate and are relatively weak swimmers, would be killed; 24-h LC_{50}'s for fish and ammocoetes ranged from 43 to 250 μg/L (Marking and Hogan 1967). The concentrations throughout the water column may have increased the exposure of both lampreys and nontarget fish to the chemical.

Peak Bayer residues in muscle of caged fish occurred 12–24 h after Bayer concentrations in water peaked (Table 2). When Dawson et al. (1982) exposed rainbow trout and largemouth bass to a nominal 50-μg/L solution of Bayer, they observed a lag preceding maximum uptake of Bayer in muscle of 8 h in rainbow trout and 12 h in largemouth bass.

We attempted to compensate for lag time in Bayer uptake in fish by correlating Bayer muscle residues from all caged fish samples at 8, 14, 24, 48, 96, and 168 h after application with

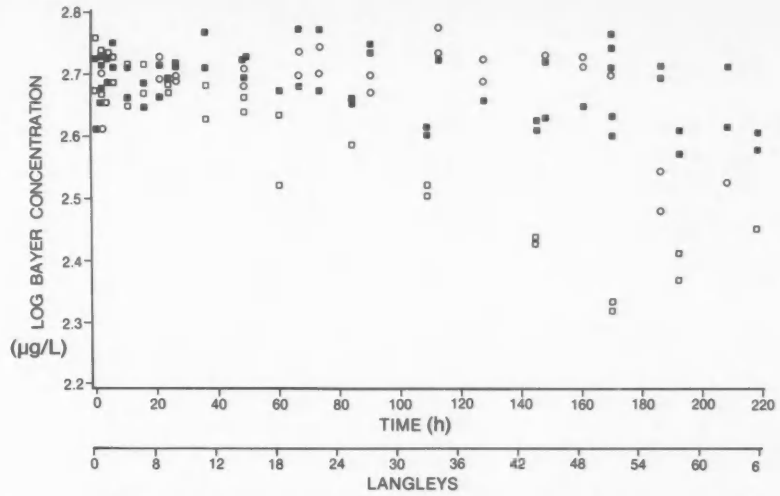

FIG. 2. Log Bayer concentration vs. time or langleys for experiment I. ■ = no sediment, sunlight, $r^2 = 0.26$; ○ = no sediment, $r^2 = 0.05$; □ = sediment, sunlight, $r^2 = 0.90$.

water concentrations at 1.5, 2, 2.5, 24, 72, and 96 h after application, respectively ($r = 0.491$ for rainbow trout, $r = 0.558$ for largemouth bass).

Correlations of fish residues and water concentrations of Bayer separated by sampling times were detected at two occasions during the first 24 h. Fish residues (8 h) were correlated with 1.5-h water concentrations ($r = 0.828$ for rainbow trout, $r = 0.915$ for largemouth bass). Similarly, 24-h fish residues correlated with 2.5-h water concentrations resulted in $r = 0.833$ for rainbow trout and $r = 0.857$ for largemouth bass. Despite evidence of overall correlations between water concentrations and fish residues of Bayer, frequent lack of correlation by station and sampling times indicated that we could not be certain how water concentrations of Bayer affected Bayer residues in the caged fish. Unknown bioaccumulation kinetics for Bayer, and rapidly fluctuating Bayer concentrations in the water column, precluded determination of cause and effect relationships over time or space.

Regression slopes for largemouth bass and rainbow trout Bayer residues from 14 to 168 h were used to examine rates of Bayer loss. No difference in elimination from muscle (slope = −1.70 for rainbow trout, slope = −1.85 for largemouth bass) was apparent. The uneven decline of residues in caged fish in Seneca Lake could reflect the temporal and spatial fluctuations in Bayer concentrations in the water column. Others have reported fluctuating muscle residues during elimination of Bayer from rainbow trout (Dawson et al. 1982; Statham and Lech 1975).

Laboratory Experiments

Experiment I: Sediment processes, photolysis, and hydrolysis

Bayer concentrations decreased at a faster rate in beakers with sediment exposed to sunlight ($N = 2$, rate constant $k = -17.20 \times 10^{-4}$ log µg Bayer·L^{-1}·h^{-1}) than in beakers without sediment exposed to sunlight ($k = -4.15 \times 10^{-4}$ log µg Bayer·L^{-1}·h^{-1} or langley equivalents, *t*-test, Fig. 2). There was no difference in disappearance rates of Bayer between light and dark beakers without sediment ($k = -10.35 \times 10^{-4}$ log µg Bayer·L^{-1}·langley^{-1}, *t*-test); therefore, the very low rates at which hydrolysis and photolysis decreased Bayer were similar. The average daily sunlight intensity (375 langleys, as estimated from September 1982 langley data) in the Seneca Lake area during early September (when Bayer was applied) was similar to the 382 langleys/d during experiment I. After 96 h, 71% of the Bayer was still present in beakers with sediment exposed to sunlight, 107% in beakers without sediment exposed to sunlight, and 110% in dark beakers without sediment.

Experiment II: Adsorption and microbial degradation

Bayer concentrations decreased faster in sterile dark test tubes with sediment ($k = -0.10677$ log µg Bayer·L^{-1}·h^{-1}) than in sterile dark test tubes without sediment ($k = +0.013571$ log µg Bayer·L^{-1}·h^{-1}, Fig. 3). The rate of Bayer loss in systems with sediment was faster over 96 h in both experiments I and II. The soil adsorption coefficient (K_{oc}) estimated from water solubility (Kenega and Goring 1978) of Bayer was 221, indicating that some adsorption to sediment was expected. Although the amount of organic carbon (0.34%) in the tested Seneca Lake sediments was low (Environmental Protection Agency (1979) recommended conducting K_{oc} measurements for soils with 1−8% organic carbon)), Lyman et al. (1982) stated that some adsorption to sediments always occurs. El-Dib and Aly (1976b) demonstrated sorption of other phenylamide pesticides to clay minerals. There was no difference in disappearance rates of Bayer among nonsterile light test tubes with sediment ($k = -0.15534$ log µg Bayer/h), sterile light test tubes with sediment ($k = -0.13734$), and sterile dark test tubes with sediment. Microbial degradation in the water column and visible light had no effect on the decline of Bayer concentration; however, sediment microorganisms could have degraded sorbed Bayer which we did not measure. El-Dib and Aly (1976c) indicated persistence of phenylamide pesticides for 4 mo in natural populations of microorganisms, although hydrolysis and metabolism has been reported for Bayer sorbed to sediments (Muir and Yarechewski 1982).

After 96 h, 12% of the Bayer remained in the nonsterile light test tubes with sediment, 29% in sterile light test tubes with

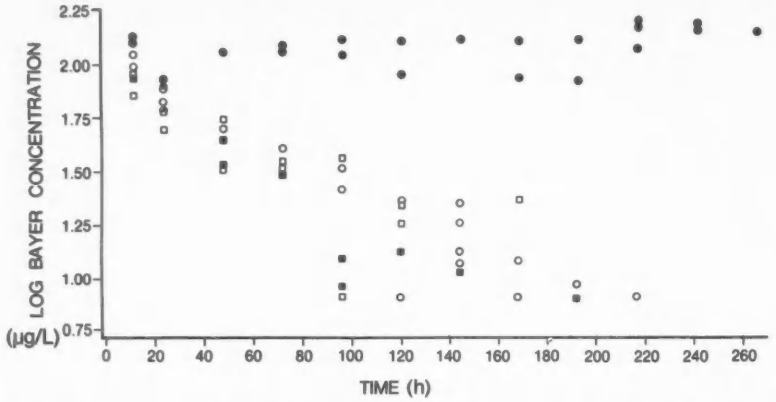

FIG. 3. Log Bayer concentration vs. time for experiment II. ■ = nonsterile, sediment, light, $r^2 = 0.86$; ● = sterile, no sediment, dark, $r^2 = 0.22$; ○ = sterile, sediment, light, $r^2 = 0.89$; □ = sterile, sediment, dark, $r^2 = 0.58$.

sediment, 22% in sterile dark test tubes with sediment, and 94% in sterile dark test tubes without sediment. While these data cannot be extrapolated directly to the environment, they do indicate the importance of sediment adsorption in the absence of dilution or dispersion opportunities. However, our lake monitoring suggested that dispersion and dissipation also accounted for a substantial portion of the loss of Bayer from the field site during the 96-h posttreatment period.

Bayer was expected to be released from granules into the bottom 5 cm of the water column. Although measured concentrations were highest in the bottom 10 cm, Bayer was released or mixed throughout the water column. Bayer concentrations varied substantially within the treatment area and may have been affected by variation in depth, application rate, or dissolution from granules. The variation was reduced when the actual application rates were weighted by volume. This actual rate varied about seven times within the treatment area from 43 to 295 mg/m^2, and bracketed the intended rate of 230 mg/m^2.

Bayer residues in muscle of caged fish reached about 800 ng/g, although concentrations were lower in most samples, and all declined to levels of 50–100 ng/g or less within 1 wk after application. These are probably worst-case estimates because fish were confined to zones where Bayer concentrations were highest. Mortality in caged fish occurred only at the site of highest Bayer levels and only in rainbow trout.

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Selection of Microhabitat in Summer by Juvenile Atlantic Salmon (*Salmo salar*)

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This study was designed to define the microhabitats selected in summer by juvenile Atlantic salmon (*Salmo salar*). Curves were developed describing the preference of 880 young salmon for water velocity at the fish's position (nose velocity), mean water column velocity, total water depth, and stream substrate size. Study sites were chosen in six morphologically diverse streams in Nova Scotia and New Brunswick during 1982–84. Of the four variables measured, only nose velocity chosen by both fry and parr was not significantly different among years or rivers. Atlantic salmon fry (<65 mm) most frequently selected nose velocities between 5 and 15 $cm \cdot s^{-1}$, small parr (65–100 mm) between 5 and 25 $cm \cdot s^{-1}$, and large parr (>100 mm) between 5 and 35 $cm \cdot s^{-1}$. Apparently, juvenile salmon utilized water depths and stream substrates which varied within tolerable limits according to their availability in conjunction with preferred water velocities. Significant differences in the body shape and size of the pectoral fin of Atlantic salmon parr in different rivers did not influence the selection of nose velocities within the range of flow conditions sampled.

Cette étude avait pour objectif la définition des microhabitats choisis l'été par les jeunes saumons atlantiques (*Salmo salar*). Des courbes de préférence en fonction de la vitesse de l'eau à la position du poisson (vitesse devant le nez), de la vitesse moyenne de la colonne d'eau, de la profondeur totale et de la granulométrie du substrat, ont été calculées avec 880 saumons. Les stations ont été choisies dans six cours d'eau à la morphologie différente, en Nouvelle-Écosse et au Nouveau-Brunswick, en 1982–1984. Des quartre variables mesurées, seule la vitesse de l'eau devant le nez choisie par le fretin et les tacons ne marquait pas de différence significative d'une année à l'autre ou d'une rivière à l'autre. Le fretin (<65 mm) choisissait le plus souvent des vitesses comprises entre 5 et 15 $cm \cdot s^{-1}$, les petits tacons (65–100 mm), entre 5 et 25 $cm \cdot s^{-1}$ et les gros tacons (>100 mm), entre 5 et 35 $cm \cdot s^{-1}$. Apparemment, les jeunes saumons choisissaient des profondeurs d'eau et des substrats qui variaient à l'intérieur de limites tolérables, selon la disponibilité et en fonction de la vitesse d'écoulement de l'eau préférée par les saumons. Les différences significatives de morphologie et de taille de la nageoire pectorale chez les tacons de différentes rivières n'ont eu aucun effet sur le choix de la vitesse de l'eau au bout du nez à l'intérieur de la plage des conditions d'écoulement échantillonnée.

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Stream discharge, particularly during the spring and summer, can substantially influence the size of Atlantic salmon (*Salmo salar*) parr populations (Frenette et al. 1984), possibly by determining a stream's depth and velocity. Water depth, water velocity, cover, and stream substrate have been shown to limit standing stocks of some warmwater fish species (Orth and Maughan 1982) and to correlate with the abundance or biomass of trout (Lewis 1969; Binns and Eiserman 1979; Stalnaker 1979). Although descriptions of juvenile Atlantic salmon habitat requirements are abundant in the literature (Saunders and Gee 1964; Elson 1967; Gibson 1978; Symons and Heland 1978; Rimmer et al. 1984), only Shirvell and Morantz (1983) and Trial and Stanley (1984) have published habitat suitability curves for this species. It is unclear from this existing information whether or not juvenile Atlantic salmon consistently choose similar microhabitats among different rivers. This determination is vital to the protection and management of salmon habitat because physical stream characteristics important to the successful rearing of young salmon must be appropriate to the population in a particular river.

In recent years, the recognition of fish habitat preferences has led to the development of habitat simulation models, particularly the "instream flow incremental methodology" (IFIM) (Bovee 1982), designed to quantify available habitat for given species of fish. The use of IFIM requires that water velocities, water depths, and stream substrates (or cover) used by a species be quantified into habitat suitability curves. The use of such curves may be valid only if they are developed from the study stream itself unless it has been demonstrated that existing curves are appropriate due to the "universality" of microhabitat selection by the species in question. The consistency of habitat selection by brown trout (*Salmo trutta*) among six streams led Shirvell and Dungey (1983) to infer that these fish choose similar habitats in all rivers. Conversely, Atlantic salmon juveniles occupied different habitats between two Maine streams (Trial and Stanley 1984), apparently because of different hab-

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TABLE 1. Physical features of the six streams sampled for juvenile Atlantic salmon microhabitat data. The Gulquac River is located in northern New Brunswick; all other streams are in Nova Scotia.

Study stream	Pembroke R.	Cox Bk.	Ohio R.	W. LaHave R.	Gold R.	Qulquac R.
Watershed area (km^2)	73.3	68.7	146.8	142.3	370	158.6
Stream length (km)	18.7	9.4	40	18	45	33
Gradient (%)	1.07	1.78	0.324	0.183	0.44	0.947
Mean annual discharge ($m^3 \cdot s^{-1}$)	2.40	2.24	4.03	3.89	11.2	3.29
Bedrock	Sandstone conglomerate	Slate	Slate granite	Slate granite	Greywacke granite	Sandstone conglomerate
Substrate	Gravel cobble	Bedrock cobble	Bedrock cobble	Boulder gravel	Boulder cobble	Gravel cobble

itat availability.

The choice of habitat by fish can be influenced by their size (Everest and Chapman 1972), their body morphology (Riddell and Leggett 1981), by both intraspecific and interspecific competition for space and food (Symons and Heland 1978; Fausch and White 1981), and by microhabitat availability (Rimmer et al. 1984). Universally selected values of a stream characteristic must be of adaptive significance to the fish and would therefore contribute to the fitness of the population.

Because it is uncertain whether juvenile Atlantic salmon have the same habitat preferences in different rivers, or at different sizes, the primary purpose of this study was to derive summer daytime habitat suitability curves for different size classes of young salmon from six physically dissimilar streams in Nova Scotia and New Brunswick. It was also our intent to determine the relative importance of water velocity, water depth, and stream substrate in the selection of microhabitat by juvenile Atlantic salmon and the possible interrelationships among these parameters. This is because implicit within IFIM is the assumption that these are the most important habitat variables and that their influence on habitat selection by fish is independent (Orth and Maughan 1982). We further set out to investigate whether possible differences in juvenile Atlantic salmon body morphology among some Nova Scotia rivers could be related to habitat selection in summer.

Materials and Methods

Juvenile Atlantic salmon microhabitat was measured through direct underwater observations of fish in the Pembroke, Gold, and Ohio rivers in 1982, in the Pembroke River, Ohio River, Cox Brook, and West LaHave River (all in Nova Scotia) in 1983, and in the Gulquac River (New Brunswick) in 1984 (Table 1). These rivers were chosen for their morphological dissimilarity (Table 1).

Sampling sites in each stream were generally 300–500 m in length but were not rigidly bounded. Each site was visited at approximately 2-wk intervals in 1982 and 1983 and monthly in 1984 from the middle of June to the end of September, so that sampling would include a wide range of summer flow conditions.

All sampling was carried out by observers wearing masks and snorkels. While most effort was directed at those locations with the highest apparent densities of fish, all areas within the sampling reach were investigated. The amount of time spent in a particular location was roughly proportional to the numbers of fish observed. We sampled water depths ranging from 8 to 150 cm. Sampling was generally carried out between 10:00 and 16:00 when underwater visibility was best.

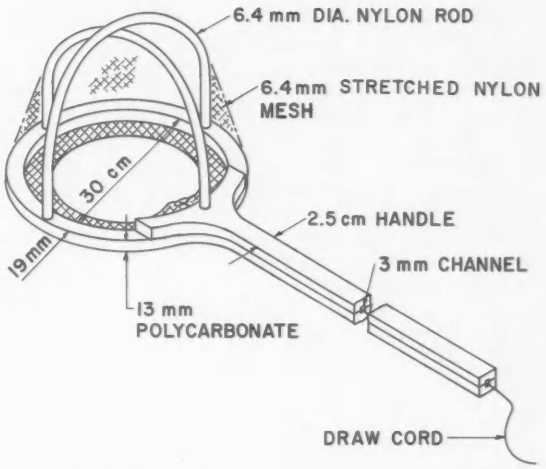

FIG. 1. Inverted hoop net used to live capture juvenile Atlantic salmon.

Fish were segregated into three (fork length) size classes: fry (<65 mm), small parr (65–100 mm), and large parr (>100 mm). These size groups conform roughly to age 0+, 1+, and 2+ parr, respectively; however, scale analysis reveals considerable overlap in length between the two older age groups.

When the observer was satisfied that an individual fish was holding a position of its own choice, the fish was captured and the exact location over the streambed was marked with a numbered lead weight to which was attached an orange float. Juvenile Atlantic salmon were caught with a trigger-activated suction gun, a spear gun, or a small-mesh inverted hoop net. The hoop nets were fitted with white, knotless, 6.4-mm stretched mesh nylon netting over a polycarbonate frame (Fig. 1). The net was lowered over the observed fish and the draw cord pulled quickly to enclose it.

The suction gun worked best for fry, while the hoop net worked best for larger fry and small parr. The spear gun, although undesirable because it killed the fish, was used for capturing large parr because the suction gun and hoop net were ineffective due to the stronger swimming ability and greater

waryness of these fish. The efficiency of the hoop net might be improved by modifying the dimensions and shape of the net opening to suit local substrate conditions and by using transparent monofilament mesh.

All captured fish were measured to the nearest millimetre (fork length) and weighed on a Sartorius model 1003 portable electronic balance to the nearest 0.1 g. At each capture location the underwater observer and an assistant measured the height of the fish above the substrate using background marks identified prior to capture, measured the total depth to the nearest 0.5 cm, and visually estimated the percentage of sand, gravel, cobble, boulder, or bedrock immediately below each fish. These substrate ratings were later coded according to the modified Wentworth particle size scale described in Bovee and Cochnauer (1977). Water velocities were measured at 0.6 of the depth below the water surface and at the fish's nose position using an Ott C2 meter fitted with a 3-cm propeller and attached to an Ott Z200 digital recorder. Velocities at these two locations correspond respectively to the mean velocity in the water column (Albertson et al. 1960) (hereafter called mean water velocity) and that experienced by a fish directly on its snout.

During 1983 a series of nine morphometric measurements were made on all captured parr. These were the characteristics suggested by Riddell and Leggett (1981) as being important in the hydrodynamics and station-maintaining ability of juvenile salmon. They included head length, width, and depth; maximum body width and depth; pectoral and pelvic fin lengths; and snout to the anterior insertion of the pectoral (SNAP) and pelvic fins (SNAPE). All measurements were made with calipers to the nearest 0.1 mm on fish anaesthetized with MS-222.

To test the assumption that Atlantic salmon juveniles select specific microhabitats within a section of stream possessing generally suitable salmon habitat conditions, we quantified stream bottom velocities and depths available in Gulquac River for each sampling day using the IFG-4 and HABTAT programs of IFIM (Bovee 1982; Milhous et al. 1984). Physical stream measurements used by these programs were carried out according to the methods of Trihey and Wegner (1981). Because we wished to quantify velocities near the stream bottom, water velocities were measured 2 cm above the substrate. We did not include substrate availability in this analysis, since virtually the entire section was composed of a gravel–cobble bottom; all fish sampled were therefore situated within this narrow range of substrates.

We used analysis of variance (ANOVA) to compare the means of microhabitat variables among rivers and between years. This test is valid only if the variances of the sample measurements are equal (Sokal and Rohlf 1969). When this condition was not met according to Bartlett's test for homogeneity of variances (Sokal and Rohlf 1969), Kruskall–Wallis and Kolmogorov–Smirnov nonparametric tests were used to compare, respectively, the distribution of microhabitat variables among rivers and between years. When variable means were significantly different, they were adjusted for fish length using analysis of covariance (ANCOVA) and again compared (Snedecor 1956). ANOVA was used to compare mean values of body measurements of young salmon among the sampled rivers. Where appropriate, the measured body characteristics were adjusted for fish length using ANCOVA in order to make allometric comparisons among fish of similar size. ANCOVA, however, was performed only when the sample regression coefficients were similar (Snedecor 1956). The F variance ratio (Snedecor 1956) provided a measure of the significance of

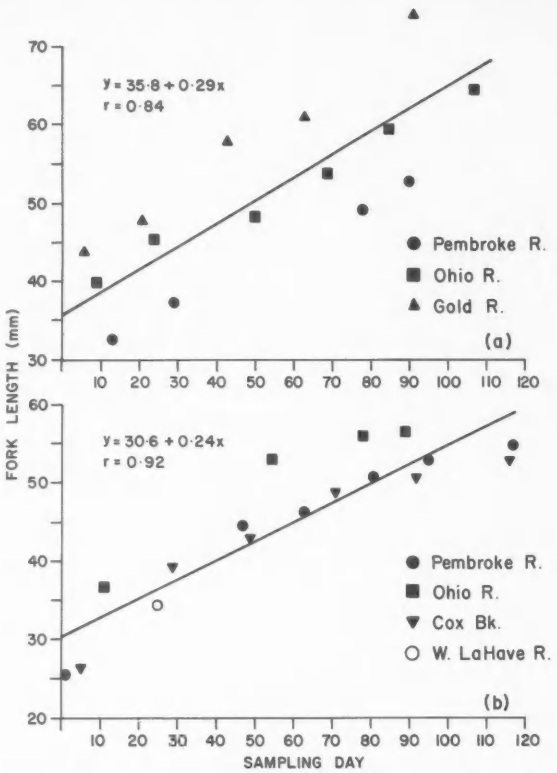

FIG. 2. Linear regressions of Atlantic salmon fry fork length against sampling day (counted from the first sampling date) in (a) 1982 and (b) 1983. June 16 and June 10 were the first sampling dates in 1982 and 1983, respectively.

regression lines and microhabitat and morphometric comparisons. To examine possible interactions among measured microhabitat variables, we used a G-test of independence in a three-way frequency table (Sokal and Rohlf 1969). Suitability indices for all microhabitat variables were developed using frequency analysis as described in detail by Bovee and Cochnauer (1977).

Habitat suitability curves are based upon the assumption that individuals of a species are not randomly distributed within an area of generally suitable habitat. Rather, they will most frequently select preferred conditions "... but will also use less favorable conditions within a defined range, with the probability of use decreasing as conditions approach the end points of the total range" (Bovee and Cochnauer 1977). The range of values of each variable determined by chi-square tests to be most frequently used was therefore considered to be optimal habitat (Bovee and Cochnauer 1977) and was assigned a value of 1.0 on the suitability scale. All other values were assigned a suitability index between 0 and 1.0 based upon their frequency of use relative to the optimum. The results of all statistical tests were judged to be significant at $P < 0.05$.

Results

The methods used to capture Atlantic salmon fry in 1982 and 1983 effectively sampled these fish at random and were not

TABLE 2. Mean values of four microhabitat variables measured in six streams in Nova Scotia and New Brunswick, 1982–84.

Year	River	Age/size class	n	Fork length (mm)[a]	Nose velocity ($cm \cdot s^{-1}$)	Mean water velocity ($cm \cdot s^{-1}$)	Depth (cm)[a]	Substrate index[a,b]
1982	Pembroke	Fry	54	41.1±10.7	10.7±6.1	38.7±23.6	31.0±13.5	5.2±0.6
		Small parr	17	77.5±9.9	12.4±13.2	28.9±28.2	53.5±27.1	4.9±0.6
		Large parr	2	109.5±12.0	15.0±7.1	26.5±14.9	36.5±9.2	5.5±0.7
1982	Gold	Fry	26	54.6±11.6	16.8±8.0	29.2±17.8	33.9±10.5	5.8±1.1
		Small parr[c]	1	94.0	12.0	30.0	36.0	5.0
		Large parr	74	128.3±17.2	24.4±12.1	35.0±19.0	49.3±13.1	6.6±0.8
1982	Ohio	Fry	50	55.3±9.2	14.4±8.5	38.0±20.7	33.9±7.8	6.0±1.2
		Small parr[c]	1	94.0	20.0	65.0	46.0	8.0
		Large parr	43	123.6±18.5	27.0±11.8	43.7±19.2	45.4±8.8	6.4±1.1
1983	Pembroke	Fry	87	47.2±9.1	12.8±8.7	33.8±18.3	41.4±14.2	5.4±0.6
		Small parr	67	80.3±6.7	16.7±10.7	37.1±22.1	57.0±24.3	5.5±0.6
		Large parr	7	102.2±2.3	26.9±10.2	44.2±27.8	52.3±21.1	6.1±0.4
1983	Cox Bk.	Fry	44	45.7±8.2	11.6±6.2	23.5±14.4	33.0±13.0	6.1±1.1
		Small parr	78	87.8±6.7	19.2±10.0	40.1±19.3	42.0±10.6	6.6±1.1
		Large parr	11	105.6±6.7	19.9±12.8	39.8±16.8	43.6±15.9	6.1±0.9
1983	Ohio	Fry	54	52.6±6.6	10.1±7.4	20.1±14.8	28.3±10.2	5.7±0.8
		Small parr	12	90.4±7.0	16.3±9.3	30.5±17.6	40.5±7.8	6.0±0.9
		Large parr	31	112.9±10.0	15.6±11.8	26.6±20.3	39.6±8.7	6.9±1.1
1983	W. LaHave	Fry	9	34.4±3.8	10.4±8.5	13.0±10.6	35.1±27.5	5.3±0.7
		Small parr	4	96.8±1.3	20.1±6.6	31.8±14.6	54.6±24.1	6.1±0.7
		Large parr	29	113.0±7.8	22.3±13.3	37.1±16.5	50.7±20.9	6.1±1.0
1984	Gulquac	Fry	87	52.4±8.7	11.3±7.5	35.7±25.6	36.9±12.5	5.3±0.6
		Small parr	60	86.7±7.8	19.8±11.2	48.2±27.3	40.0±13.3	5.7±0.6
		Large parr	32	115.7±13.3	13.0±6.8	25.1±17.8	46.6±12.4	5.9±1.1
Overall means		Fry	411	49.2±10.3	12.1±7.9	31.6±21.2	35.1±13.1	5.6±0.9
		Small parr	240	85.0±8.3	19.0±10.7	39.9±23.2	46.7±19.0	5.9±0.9
		Large parr	229	119.9±16.4	21.6±12.4	34.8±19.8	46.8±13.8	6.4±1.0

[a]Mean values ± standard deviation.
[b]Derived from the modified Wentworth particle size scale (see Fig. 3).
[c]Data are provided for the one fish caught in this size class.

TABLE 3. Regression coefficients, correlation coefficients, F-distributions, and probabilities resulting from linear regressions of four microhabitat variables on Atlantic salmon fry and parr fork lengths. *Statistically significant regression.

	Regression of fish length with:	a	b	r	F	P
Fry	Nose velocity	5.2	0.14	0.17	11.25	<0.001*
	Mean water velocity	50.1	0.01	0.03	0.01	0.75
	Depth	31.6	0.07	0.05	1.39	0.25
	Substrate	4.3	0.03	0.31	42.93	<0.001*
Parr	Nose velocity	4.8	0.15	0.31	40.67	<0.001*
	Mean water velocity	37.1	0.00	0.00	0.02	0.75
	Depth	49.9	−0.02	0.04	−0.66	0.40
	Substrate	4.7	0.02	0.37	59.92	<0.001*

biased towards fry of any particular size, as evidenced by the strong linear relationship in both years between fork length and time ($P < 0.001$) (Fig. 2). This was substantiated in 1984 when fry sampled from the Gulquac River showed significant growth over the summer period ($n = 87$, $r = 0.90$, $P < 0.001$).

However, Atlantic salmon parr were not captured at random. This was apparent from the lack of significant seasonal growth in our parr samples in any of the three years of this study. The spear gun, used predominantly to catch parr in 1982, was selective for large fish as indicated by the paucity of small parr in that year's sample (Table 2). Conversely, the trap net, used in 1983 and 1984, was inefficient at capturing larger fish which tended to be more wary and swim faster. When data from the three years (and hence from the different sampling methods) were pooled for both small and large parr, fish length became significantly associated with time (measured from the date of first sampling in each year) ($n = 309$, $r = 0.13$, $P = 0.02$ for small parr; $n = 229$, $r = 0.23$, $P < 0.001$ for large parr). A combination of sampling methods may therefore representatively sample the entire population.

The regressions of nose velocity and substrate on fish length for fry and parr (data for both years and both parr size classes were pooled) were highly significant (Table 3). Juvenile Atlantic salmon occupied faster water over larger substrates as they grew. However, there is no indication that larger fish within each size class necessarily moved into deeper water (Table 3). This is substantiated by the similiarity in mean depths occupied by small and large parr (Table 2) ($F = 0.03$, $P = 0.86$). Fry, however, preferred depths on average about 14 cm shallower than parr ($F = 51.2$, $P < 0.001$). It is apparent from the lack of any significant association between mean water column velocity and fish length (Table 3) that fry and parr select their stream position at least in part on the basis of water velocities near the bottom. The significant relationship between both fry and parr nose velocities and mean water velocities ($n = 706$, $r = 0.53$, $F = 263.0$, $P < 0.001$) does imply, however, that larger fish would often coincidentally assume a position near faster surface currents.

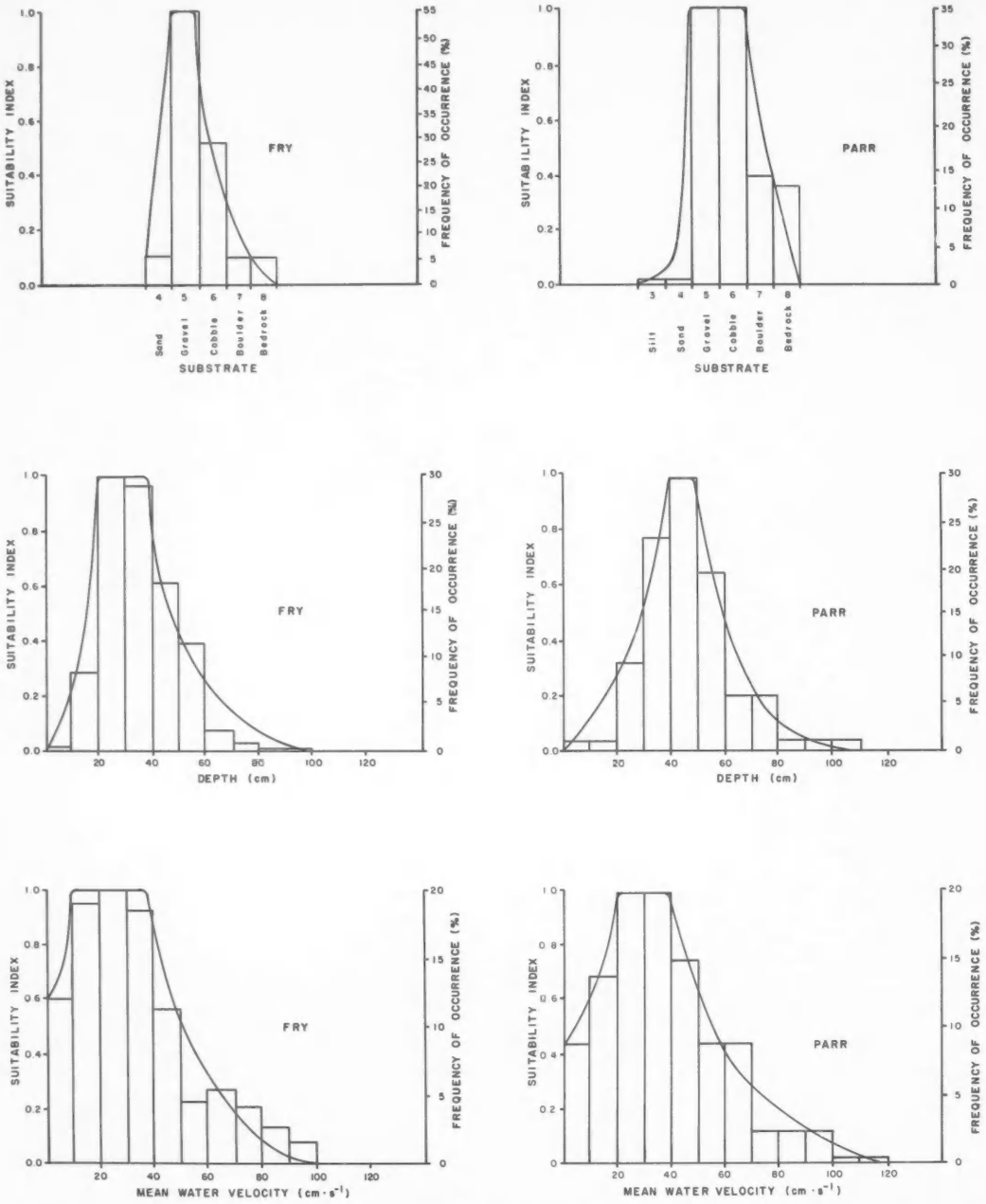

FIG. 3. Substrate, total water depth, and mean water velocity (measured at 0.6 of the depth from the water surface) selection by Atlantic salmon fry and parr in six Nova Scotia and New Brunswick rivers in 1982–1984. Bars represent frequency of occurrence; curves indicate the suitability index.

Mean nose velocities chosen by fish of equivalent length within each size group (fry, small parr, and large parr) were similar when compared among rivers and years ($P = 0.14$). However, large parr in the Gulquac River were, on average, sampled in significantly slower bottom velocities than those in Nova Scotia (13 vs. 23 $cm \cdot s^{-1}$; $F = 19.4$, $P < 0.001$). This can be attributed to the fact that only 32 large parr were caught in the Gulquac River and the majority of these were from a side-channel pool with little current. Many other large parr were observed in the Gulquac River in microhabitats visually similar to those we sampled (mainly by spear gun) in the Nova Scotia streams but the trap net was not efficient at sampling

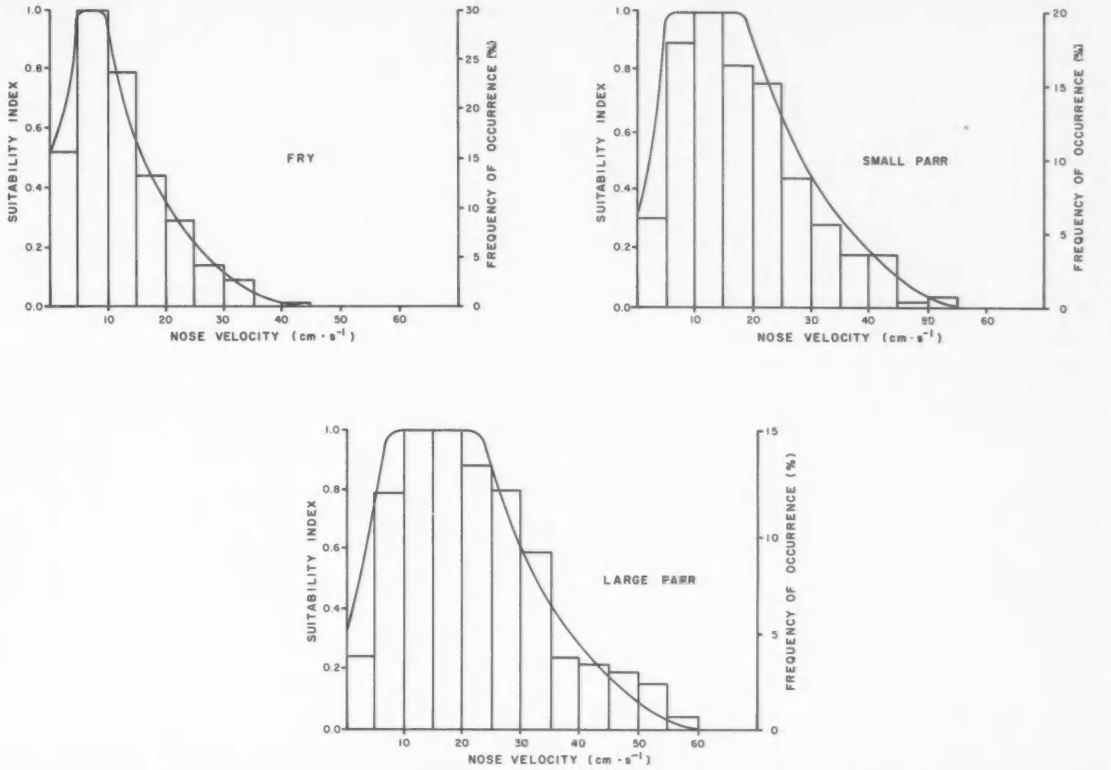

FIG. 4. Water velocities at the fish's nose position selected by fry (<65 mm), small parr (65–100 mm), and large parr (>100 mm) in six Nova Scotia and New Brunswick rivers in 1982–1984. Bars represent frequency of occurrence; curves indicate the suitability index.

these fish.

Juvenile salmon sampled during this study did not select uniform depths, substrates, or mean water velocities from river to river or year to year (Table 2) as they did with nose velocities. Such variation in habitat utilization would not likely have been encountered if only a single stream had been sampled. The suitability curves assembled from the pooled microhabitat data (from all rivers) (Fig. 3 and 4) therefore represent the range of habitat juvenile Atlantic salmon will use across a variety of conditions they might normally have to contend with throughout their distribution.

Since no significant differences in mean water velocities, depths, and substrates were detected between small and large parr (ANOVA, $P > 0.5$), the data for these parameters were also pooled to produce suitability curves applicable to all parr (Fig. 3). The creation of separate nose velocity curves for small and large parr (Fig. 4) was dictated by the fact that large parr chose significantly faster currents in which to maintain position (ANOVA, $P < 0.001$). This choice of velocities is apparently governed by fish size; when small and large parr lengths were standardized using ANCOVA, the selection of nose velocities was shown to be similar ($F = 0.7$, $P = 0.4$).

The nose velocity suitability curves for fry, small parr, and large parr (Fig. 4) demonstrate that as young salmon grow they are capable of coping with considerably faster flows and simultaneously make decreasing use of low-velocity areas. Still, there is substantial overlap in velocities occupied by juvenile salmon, particularly in the 5–10 $cm \cdot s^{-1}$ range. Optimal nose velocities range from 5 to 10 $cm \cdot s^{-1}$ for fry, 7–15 $cm \cdot s^{-1}$ for small parr, and 10–20 $cm \cdot s^{-1}$ for large parr. Fry frequently utilize zones of slow flow (0–5 $cm \cdot s^{-1}$), particularly at stream edges or behind large cobbles. The areas of reduced velocity selected by parr were generally in pools.

The shapes of the suitability curves for mean water velocity (Fig. 3) likely result from the coincidental linear relationship between nose velocities and mean water velocities. The selection of faster water near the stream bottom would therefore situate a fish near swifter midwater currents. No juvenile salmon were found in locations where the mean water velocity exceeded 120 $cm \cdot s^{-1}$. Such conditions probably create excessive turbulence which would force individual fish to seek locations with more tolerable water currents. Some Atlantic salmon parr (26 fish) were sampled in pool areas with reduced mean water velocities. Conversely, fry were seldom found in pools; water velocites above fry sheltering behind cobbles were usually relatively rapid.

Salmon fry and parr most frequently selected depths between 20 and 40 cm and 30 and 60 cm, respectively (Fig. 3). For all fish sizes, selected depths did not exceed about 100 cm and were rarely less than 15 cm.

Salmon parr were found equally over gravel and cobble, while fry were primarily associated with gravel stream bottoms (Fig. 3). Substrate particle size is related to water velocity, since faster flows are capable of transporting larger materials

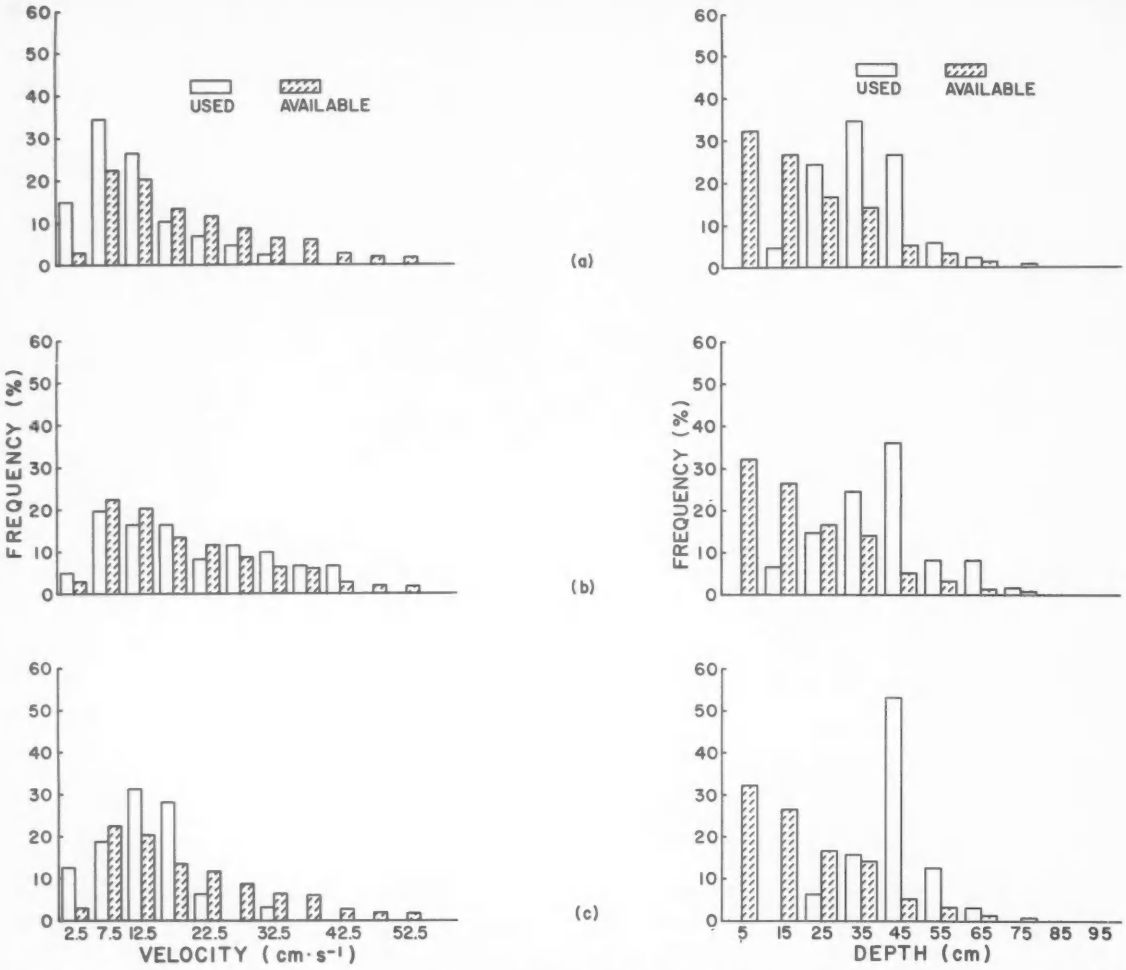

FIG. 5. Water velocities at the fish's nose position and total water depths selected by Atlantic salmon (a) fry (n = 87), (b) small parr (n = 60), and (c) large parr (n = 32) and the distribution of bottom water velocities and total water depths available in the Gulquac River sampling site at four different discharges in 1984.

(Dunne and Leopold 1978). It is probably true, therefore, that fry predominate over gravel, since cobble occurs mainly in higher velocity zones in the stream channel. Although the pooled data show only limited use of boulder and bedrock locations by young salmon, 27% of the fry and 39% of the parr (all but one were small parr) sampled in Cox Brook were found over these substrates. The slate bedrock in Cox Brook has a jagged surface with many crevices which might shield fish from excessive velocities. This is substanstiated by the fact that fry and parr found over bedrock faced mean nose velocities of 11.6 ± 5.4 cm·s^{-1} (n = 12 fry) and 17.7 ± 9.0 cm·s^{-1} (n = 23 small parr), respectively, values not significantly different ($P > 0.8$) from either the Cox Brook means or the grand sample means (Table 2).

Fry and parr in the Gulquac River were not distributed in proportion to the bottom velocities and depths available to them (Fig. 5), attesting to the preference of juvenile Atlantic salmon for specific values of these physical parameters. We assume that these fish also show preferences for particular substrates, since they were found almost exclusively over stream bottom material larger than sand despite the occurrence of zones of finer particles in some of the streams sampled.

The G-test of independence of nose velocity, depth, and substrate indicated no significant interactions among these variables for fry (G = 143.7, df = 340, $P > 0.5$) and parr (G = 376.8, df = 428, $P > 0.5$). These physical characteristics appear to influence independently the selection of habitat by young salmon.

The nine morphological body characteristics measured from small and large parr in 1983 (summarized in Table 4) were all highly linearly related to fish length ($P < 0.001$ in all cases). For this reason and because parr lengths differed significantly among the four Nova Scotia rivers (F = 66.8, $P < 0.001$), it was important to make allometric comparisons among fish of equivalent size. We therefore tested the differences in the means of each morphological characteristic using ANCOVA

TABLE 4. Mean values (±SD) of 10 morphometric characteristics measured from small and large salmon parr in four Nova Scotia rivers (sample sizes in parentheses).

Mean morphometric measurements (mm SD)	Pembroke R. (57)	Cox Bk. (48)	Ohio R. (38)	W. LaHave R. (30)
Fork length	84.4±9.0	90.3±7.7	107.3±13.8	110.6±9.9
Head length	20.7±2.2	21.5±2.0	24.5±3.9	26.8±4.4
Head width	8.7±1.1	9.1±1.4	11.4±2.4	11.9±2.0
Head depth	11.0±1.3	11.7±1.2	14.1±1.8	15.3±2.3
Max. body width	8.2±1.6	9.0±2.1	12.0±2.2	12.6±1.1
Max. body depth	14.0±2.4	15.1±2.4	20.4±3.1	21.7±2.8
Pectoral fin length	18.2±1.7	19.3±1.7	21.0±2.5	21.7±2.8
Pelvic fin length	12.4±1.3	13.3±1.2	14.8±1.9	15.5±1.6
SNAP[a]	18.8±1.6	19.6±1.9	22.7±3.2	23.5±2.3
SNAPE[b]	41.9±4.6	44.5±3.8	51.7±6.8	53.7±5.1

[a]Snout to the anterior insertion of the pectoral fin.
[b]Snout to the anterior insertion of the pelvic fin.

(with fork length as the covariate) on all combinations of paired rivers. Mean body measurements were not significantly different between Pembroke River and Cox Brook parr (P ranged from 0.2 to 0.9), and only head depth varied significantly between Ohio River and West LaHave River parr ($F = 4.4$, $P = 0.04$; all other probabilities ranged from 0.06 to 0.6). Pembroke River and Cox Brook parr body morphology data were therefore pooled as they were for the Ohio and West LaHave rivers. Allometric comparisons between these two data sets were made using ANCOVA (Table 5).

Differences in body morphologies did not influence microhabitat selection (with respect to velocity), at least under the range of flow conditions sampled. Although the Ohio River and West LaHave River fish had consistently larger head and body measurements than Pembroke River and Cox Brook parr of equivalent length, these latter fish possessed significantly longer pectoral fins (Table 5). Pembroke River and Cox Brook parr, with more streamlined body shapes and larger pectoral fins, might be better adapted than fish in the other two rivers to maintain position in fast currents. While this may be true, there were no significant differences among these rivers in the nose velocities chosen by fish of equivalent length (ANCOVA, $F = 2.2$, $P = 0.09$).

Discussion

The juvenile life stage of the Atlantic salmon has been described as one primarily concerned with feeding and growth (Wankowski and Thorpe 1979). It is to be expected, then, that across their range these fish would occupy habitats which permit efficient utilization of available food while minimizing the expenditure of energy.

Numerous published accounts commonly describe juvenile Atlantic salmon lotic habitat as relatively shallow, moderately fast-flowing riffle sections of streams over coarse substrates (Keenleyside 1962; Saunders and Gee 1964; Elson 1967; Symons 1976; Symons and Heland 1978; Wankowski and Thorpe 1979; Rimmer et al. 1983, 1984). In streams, these fish probably adopt similar microhabitat selection strategies throughout their geographic distribution.

The results of our study tend to support the assumption of universality of water velocity selection. Young salmon of equivalent size preferred similar water velocities near the stream bottom in all study sites. However, significant differences in the selection of depths and substrates existed among the rivers. We consider this an indication that young salmon can tolerate a relatively wide variety of depths and substrates but that water velocity near the stream bottom is the dominant physical factor influencing the selection of position (microhabitat) by juvenile Atlantic salmon. For example, Cox Brook fry and parr sampled from positions over bedrock (a substrate not commonly associated with salmon rearing) selected similar nose velocities to those chosen by fish in more typical gravel–cobble locations.

The microhabitat suitability curves provided in Fig. 3 and 4 could be useful for habitat assessment and for the restoration or improvement of juvenile Atlantic salmon rearing habitat. But, the major application of these curves will probably be for the habitat prediction model, IFIM. This model assumes that depth, velocity, and substrate independently influence microhabitat selection (Orth and Maughan 1982). This assumption has been demonstrated to be invalid under some conditions for macroinvertebrates (Gore and Judy 1981), brown trout (Shirvell and Dungey 1983), and some warmwater fishes (Orth and Maughan 1982). However, the assumption of independence has been satisfied for juvenile Atlantic salmon in this study.

In the lotic environment, the nose velocity, depth, and substrate curves are valid descriptions of summer juvenile Atlantic salmon microhabitat selection throughout the distribution of this species. The concept of universality of habitat selection by certain fish is not new. Shirvell and Dungey (1983) suggested that this may be true for brown trout, based upon their observations in six New Zealand streams. However, our assumption that our habitat suitability curves are applicable over the freshwater range of Atlantic salmon applies only in streams. Evidence is available that where predation or competition by other fish species is not a significant factor, salmon parr readily occupy lakes (Pepper 1976; Pepper et al. 1985). Further, Gibson (1978) and Gibson and Power (1975) reported changes in the habitat occupied by both Atlantic salmon parr and brook trout (*Salvelinus fontinalis*) when one or the other of the species is absent. We suggest, therefore, that juvenile Atlantic salmon do not exhibit complete universality of habitat selection. Rather, predation and competition modify habitat selection.

Juvenile Atlantic salmon spend much of their time during the summer maintaining position over the substrate in moderate currents and periodically darting out into faster water to capture prey. They are able to do so without expending excessive energy by streamlining and the development of large pectoral

TABLE 5. Comparison of adjusted mean values[a] of nine morphological characteristics of juvenile Atlantic salmon parr between Pembroke River/Cox Brook pooled data and Ohio River/West LaHave River pooled data, using ANCOVA.

Morphological characteristic	Pembroke R./Cox. Bk. adjusted mean	Ohio R./W. LaHave R. adjusted mean	*F*	*P*
Head length	22.7±0.2	23.0±0.3	0.4	0.55
Head width	9.6±0.1	10.7±0.2	10.7	0.001
Head depth	12.3±0.1	13.1±0.1	9.5	0.002
Max. body width	9.2±0.2	11.2±0.2	28.7	<0.001
Max. body depth	16.1±0.2	18.5±0.2	33.5	<0.001
Pectoral fin length	20.2±0.1	19.0±0.1	18.3	<0.001
Pelvic fin length	13.8±0.1	13.5±0.1	1.8	0.18
SNAP[b]	20.8±0.1	20.7±0.2	0.1	0.75
SNAPE[c]	47.1±0.2	46.4±0.2	3.5	0.06

[a]Means were adjusted to values for fish of equivalent length.
[b]Snout to the anterior insertion of the pectoral fin.
[c]Snout to the anterior insertion of the pelvic fin.

fins used as hydrofoils (Riddell and Leggett 1981). In their comparison of Atlantic salmon morphometrics between two New Brunswick streams, Riddell and Leggett (1981) found that streamlining and pectoral fin size were directly related to current velocity. Increased abilities to maintain position in fast flows would be particularly advantageous in streams which have either high gradients or relatively few hiding places. It might have been expected that, because of their increased streamlining and larger pectoral fins, Pembroke River and Cox Brook parr would have selected generally faster water velocities than fish in the Ohio and West LaHave rivers. That we did not find this is likely an indication that the body morphology of juvenile salmon is the result of adaptation to long-term flow conditions and other physical characteristics of these streams. Pembroke River and Cox Brook are considerably steeper than the other two rivers (Table 1), indicating that they probably have greater velocities, particularly during high water.

Interpopulation differences in body morphology of juvenile Atlantic salmon likely have a genetic basis (Riddell and Leggett 1981). It is therefore important that Atlantic salmon stocking strategies include consideration of the habitat requirements and tolerances of hatchery-reared fish.

Our microhabitat data, particularly the nose velocity suitability optima, differ substantially from those measured in a New Brunswick stream by Rimmer et al. (1984). Those authors found fry predominantly in velocities of 10–20 $cm \cdot s^{-1}$, age 1+ fish (5.0–10.0 cm in length) in velocities of 20–40 $cm \cdot s^{-1}$, and age 2+ parr (7.5–13.0 cm) in velocities of 30–50 $cm \cdot s^{-1}$, all considerably higher than the nose velocities selected most frequently by fish in our study. This discrepancy is made even more apparent by the fact that juvenile salmon in both studies were similarly located in ventral contact with the stream bottom (Rimmer et al. 1983). Rimmer et al. (1984) also measured surface and maximum water velocities associated within 1 m of positions occupied by fish in each age class. The predominant nose velocities selected by fry and parr during summer closely resembled surface water velocities and were nearly as fast as the maximum water velocities measured within 1 m of these fish. This occupation of relatively fast velocities contradicts the hypothesis that salmonids generally maintain position in low velocities near the stream bottom except when they dart into faster currents to defend territories or intercept drifting prey (Everest and Chapman 1972; Fausch and White 1981; Fausch 1984).

Rimmer et al. (1984) measured water velocities using midget Bentzel current speed tubes (Everest 1967) which, because they are hand held, require the underwater observer to be close by. Morantz et al. (1986) found that a snorkeler located within 0.5 m of a current meter significantly influenced water velocities measured near the stream bottom. They further reported that an individual positioned in the water at right angles to the flow tended to increase velocities at the meter.

It is therefore possible that the technique Rimmer et al. (1984) used to measure nose velocities overestimated velocities faced by juvenile Atlantic salmon. Using methods and equipment similar to ours, de Graaf and Bain (1987) found that Atlantic salmon parr in a Newfoundland stream chose velocities statistically similar to those documented in the present study. This strengthens our assumption that the suitability curves presented earlier have general application in typical Atlantic salmon stream habitat.

It is probable that young Atlantic salmon choose stream locations predominantly on the basis of velocities near the stream bottom due to the adaptive significance of being located in areas where potential profit and hence fitness are maximized. Juvenile Atlantic salmon in our study apparently adopted the same strategy as chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) (Everest and Chapman 1972) of occupying low-velocity areas near the stream bottom even where midstream velocities were swift. Their concurrent finding that water velocities were positively correlated with amounts of insect drift led Everest and Chapman (1972) to conclude that the occupation of sheltered locations at the stream bottom in close proximity to high velocities (and consequently substantial food drift) would minimize the energy expenditure associated with maintaining position while maximizing food availability. The potential for net energy gain (potential profit) is related to specific growth rate, which Fausch (1984) suggested is correlated with fitness in fish; individuals that grow fastest can invest more energy in reproduction and ultimately produce larger numbers of offspring.

Within an IFIM application, we recommend the use of stream bottom velocities and nose velocity suitability curves for predictions of juvenile Atlantic salmon habitat and for habitat predictions of any species occupying territories near the substrate in running water. The use of mean water velocities would likely lead to habitat overestimation. This is because these fish occupy only a fraction of the total area of a stream

reach, in pockets of preferred velocities among rubble on the stream bottom.

Acknowlegements

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A Simple Method for Estimating the ^{15}N Content of Dissolved Organic Matter ($DO^{15}N$) in N-Cycling Studies

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Axler, R. P., and J. E. Reuter. 1987. A simple method for estimating the ^{15}N content of dissolved organic matter ($DO^{15}N$) in N-cycling studies. Can. J. Fish. Aquat. Sci. 44: 130–133.

A method is described for isolating the total pool of dissolved organic nitrogen (DON) from oligotrophic lake water for subsequent isotopic analysis of the $^{15}N/^{14}N$ ratio in tracer experiments. The procedure involves reducing an acidified filtered sample with elemental zinc to convert nitrate + nitrite to ammonium and then alkalyzing and sparging with N_2 to remove ammonia. The residue (DON) is evaporated to dryness, rehydrated, spotted on a filter, and vacuum ampulated for micro-Dumas conversion to N_2 and isotopic analysis by optical emission spectrometry. We have used the method to accurately estimate ^{15}N enrichmentrs of <1 atom-% in DON collected from 150-mL samples of [^{15}N]nitrate-enriched mesocosms in Castle Lake, California.

Une méthode est décrite pour isoler le pool total de l'azote contenu dans les matières organiques dissoutes (N-MD) dans l'eau de lacs oligotrophes, afin d'obtenir le rapport $^{15}N/^{14}N$ par dosage isotopique dans les tests avec marqueurs. Il faut réduire avec du zinc élémentaire un échantillon filtré et acidifié pour convertir le nitrate et le nitrite en ammonium, ensuite alkyler et faire barboter avec du N_2 pour extraire l'ammoniac. Une évaporation est pratiquée jusqu'à ce que le résidu (N-MD) soit sec; ce dernier est ensuite réhydraté, déposé par gouttes sur un filtre et placé dans des ampoules sous vide; une conversion micro-Dumas en N_2 et un dosage isotopique par spectrométrie d'émission optique sont ensuite pratiqués. Nous avons utilisé la méthode pour doser avec exactitude des échantillons enrichis de ^{15}N de <1 atome-% du N-MD obtenu à partir d'échantillons de 150 mL de mésocosmes enrichis de [^{15}N]nitrate dans Castle Lake, Californie.

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Freshwater and marine phytoplankton living in nitrogen-impoverished waters generally depend on recycled ammonium to meet their cellular demands for growth (Dugdale 1976; Eppley and Peterson 1979; Axler et al. 1981, 1982). In these systems a quasi-steady state exists in which the ambient pool of NH_4^+ remains extremely low and relatively constant due to the balance between uptake and regeneration processes. Regenerated N is supplied by zooplankton excretion and bacterial mineralization in the water column and by the diffusive flux of inorganic and organic N from bottom sediments. The most sensitive and accurate techniques for measuring rates of regeneration have used ^{15}N isotope dilution methodology (e.g. Alexander 1970; Harrison 1978; Caperon et al. 1979; Glibert et al. 1982; Fisher and Morrissey 1985). While isotope dilution models are useful for calculating both NH_4^+ uptake and regeneration, Laws (1984) has pointed out that these measurements could be in error due to nitrification, adsorption of ammonium ions to clay particles (both colloidal and silt-sized) or to the reaction flask, and (in particular) release of dissolved organic nitrogen (DON) during the incubation period. This suggests that under certain conditions it may be important to maintain a more complete ^{15}N inventory during uptake experiments (Laws 1984; Fisher and Morrissey 1985). Using this approach, the experimenter could not only obtain a more accurate estimate of ^{15}N uptake and regeneration but also directly determine rates of other potentially important N transformations.

In the vast majority of inorganic ^{15}N uptake studies the production of $DO^{15}N$ has been totally ignored. Although methodological artifacts such as the passage of ultraplankton and colloidal material or cell lysis during filtration could cause an increase in the $DO^{15}N$ pool, natural processes such as release by phytoplankton, periphyton, bacteria, and zooplankton may also occur. Indeed, Schell (1974) and Chan and Campbell (1978) found that in natural systems, 10–15% of [^{15}N]nitrate additions were recovered in the amino acid and/or peptide fractions of the total DON pool. In this paper we present a relatively simple and inexpensive technique for isolating DON from natural lake water and measuring its ^{15}N enrichment.

Two general approaches are possible. The first involves the isolation and collection of specific fractions of DON (e.g. amino acids, peptides, proteins) using molecular sieves, ion-exchange chromatography, or HPLC techniques (Schell 1974; Chan and Campbell 1978). The second is based on the removal of dissolved inorganic nitrogen (DIN) from the pool of total dissolved N leaving behind only the DON fraction. If this method is chosen, it is essential that NO_3^-, NO_2^-, and NH_4^+ be completely removed from the sample in order to avoid contamination. We used the latter approach to provide estimates of the flux of ^{15}N from nitrate to DON in mesocosm enclosures in Castle Lake, California. This is an oligotrophic lake at which the relationship between N availability and primary production

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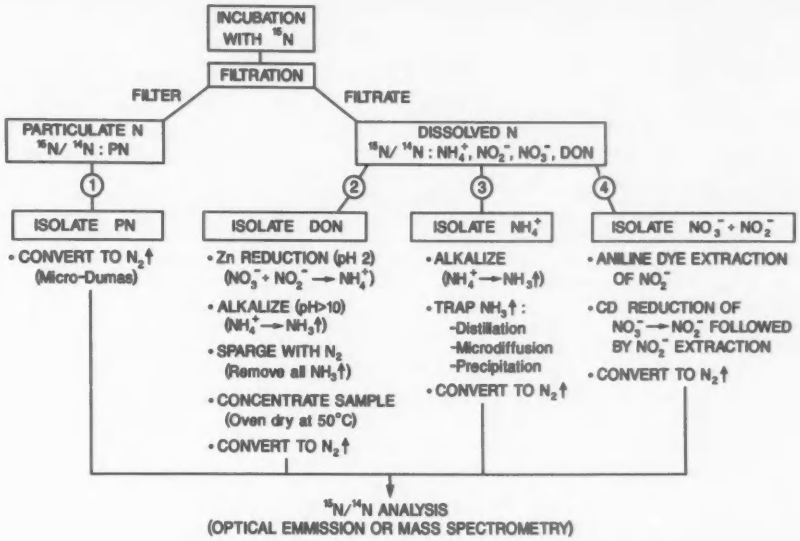

FIG. 1. Diagram of various procedures used to isolate N fractions for isotopic analysis in ^{15}N-cycling studies. Pathway 2 summarizes the method described in this report. Alternative procedures for isolating specific portions of the DON pool are cited in the text. Specific references for pathway 3 are Fiedler and Proksch (1975) and Fisher and Morrissey (1985) and for pathway 4 are Schell (1974) and McCarthy et al. (1984). Collection of N_2 gas (*re*-dinitrogen fixation and denitrification) is not included for simplicity's sake.

has been an important research focus for over 20 yr (Goldman and de Amezaga 1984).

Materials and Methods

A comprehensive flow diagram depicting the procedures for the measurement of ^{15}N enrichment in the soluble pool is presented in Fig. 1. A detailed description of our method (pathway 2) is presented below. ^{15}N uptake experiments were terminated by filtering the lake water through a precombusted GF/C glass fiber filter. At this point the filtrate can either be frozen or processed immediately. The filter can be saved to measure uptake of the ^{15}N label by the plankton (pathway 1).

The initial step in isolating the DON fraction involved the reduction of $NO_3^- + NO_2^-$ in the filtrate to NH_4^+. The filtrate was acidified to pH 2 with Ultrex H_2SO_4 (J. T. Baker Chemical Co.) to avoid nitrogen contamination. Then it was passed through a glass column (10 × 0.7 cm) packed with elemental zinc (30 mesh granules, Stainton et al. 1977). The sample volume depends on the ambient DON concentration and the amount of N needed for each $^{15}N^{14}N$ analysis (e.g. ~10 μg of N for emission spectrometry). Reduction efficiency is dependent on the pH and the residence time in the zinc column (M. Stainton, Freshwater Institute, Winnipeg, Man. R3T 2N6, pers. comm.). For Castle Lake water (alkalinity of ~200 μeq·L^{-1}), 1.0 mL of a 4 *N* solution was sufficient for each 250 mL of sample. The reaction between the acid and zinc results in the evolution of nascent H_2 which can impede the flow through a gravity column; therefore, it was necessary to use a peristaltic pump (Masterflex) fitted with tygon tubing. A flow rate of 1.3 mL·min^{-1} was sufficient for the specifications of our column. The zinc reduction column was thoroughly flushed with 2 *N* acid before samples were introduced. The expected column life is at least fifty 250-mL samples.

A series of tests was conducted to quantify the efficiency of this reduction step using both filtered lake water (from Castle Lake and Lake Tahoe) and deionized water (DI water) enriched to a final NO_3^-−N concentration of 30−80 μg N·L^{-1}. In all cases the conversion was virtually complete (98 ± 1%, $\bar{x}$ ± SD, n = 4) and no difference was observed between the DI water and natural samples. We used the phenol−hypochlorite method for NH_4^+ determinations and cadmium reduction/NED-sulfanilimide for $NO_3^- + NO_2^-$ (American Public Health Association 1980). It is important to note that the release of ionic zinc could interfere with these chemical analyses. In fact, we were unable to use our laboratory's routine nitrate procedure, hydrazine reduction to nitrite, because of apparent zinc interference. Spike recovery tests conducted on samples passed through the zinc column indicated nearly complete recoveries (>95%) of nitrate, nitrite, and ammonium using the methods cited above. At this stage the filtrate contains only ammonium and DON.

To remove the $^{14/15}N$-labelled ammonium from the filtrate, the pH was raised to 10.2−10.8 using a 4 *N* solution of NaOH (Mallinkrodt No. 7708) and sparged with N_2 gas at room temperature. An insoluble, white zinc hydroxide precipitate formed immediately and had to be filtered through a precombusted, acid-rinsed GF/F glass fiber filter. To ensure that dissolved organic matter was not deaminated, trapped in the zinc column, or lost due to the removal of the precipitate, solutions of filtered lake water spiked with glycine in the range of 0.2−10 ppm C were run through the entire procedure. Subsamples were removed after each step and analyzed for DOC and DON using a Xertex Dohrman total carbon analyzer

TABLE 1. Comparison of oven-drying (50°C) and freeze-drying (−30°C) for concentrating water samples prior to $^{15}N/^{14}N$ emission spectrometry. Mesocosms were polyethylene tubes (~4 m^3) installed in Castle Lake and enriched with 38 μg $^{15}NO_3^-$-N·L^{-1}. The initial enrichment of nitrate was ~90 atom-% ^{15}N, and ambient DON levels were ~73 μg N·L^{-1}. Therefore, the ^{15}N content of the DON pool represented <2% of the initial nitrate amendment.

Sample	Atom-% ^{15}N enrichment of DON		
	Oven-dried	Freeze-dried	% difference
Lake mesocosm No. 1 (day 18)	0.703	0.730	3.7
Lake mesocosm No. 2 (day 18)	0.644	0.786	19.9
[^{15}N]glycine standard	5.65	5.57	1.4

(persulfate/UV combustion) and a LaJolla Scientific UV oxidizer, respectively. The results indicated that neither deamination nor loss of organic matter exceeded 10%.

At a pH > 10, most of the ammonia-N exists as gaseous NH_3 (the pK_a for NH_3/NH_4^+ is 9.2). Provided that the pH of the solution remains high, the NH_3 can be quantitatively removed from solution by steam distillation (American Public Health Association 1980) or simply by sparging at room temperature with an inert gas. The reduced, processed sample was placed in a side-armed Erlenmeyer flask and sparged with N_2 gas which was bubbled through a sintered glass frit at a rate of 300−400 mL·min^{-1}. Simultaneously, a vacuum was applied to the sidearms to aid in the removal of NH_3. The efficiency of ammonia removal was tested, again using NH_4^+-enriched natural lake samples and deionized water at a final concentration of 100 μg N·L^{-1}. The mean percent removal was 97 ± 3 (n = 5); however, in all cases at least 40 h of sparging was necessary to achieve this level of reduction. The combined efficiency for nitrate reduction and ammonia removal was 94%. This value could be increased by the use of a longer zinc column or a lower flow rate. In addition, during the evaporation step (discussed below) the final traces of ammonia are volatilized, thereby increasing the final DIN removal efficiency to at least 98%.

The remaining filtrate now contains only $DO^{14/15}N$. To prepare the sample for ^{15}N analysis the solution was either freeze-dried or evaporated in a drying oven at 50°C in dichromate-cleaned, 475°C-baked beakers, rehydrated with 1 mL of deionized water, and spotted (100 μL at a time) onto a pre-combusted, acid-rinsed GF/C filter. We found little difference between these two concentration methods using both a [^{15}N]glycine standard and an actual lake water sample taken from a $^{15}NO_3$-enriched mesocosm enclosure (Table 1). While a quantitative transfer of the rehydrated solution is not critical, the final amount of nitrogen deposited on the filter must be in the range required for emission or mass spectrometry. We chilled the rehydrated residue during the transfer process to avoid changes in the DON concentration which would be caused by evaporation. If the residue (i.e. natural dissolved compounds in lake water and salts from the acid base additions) does not go into solution with the deionized water, Ultrex HCl (to a final concentration of 0.5 N) can be added. In our study the percent ^{15}N enrichment was measured using a Jasco N-150 optical emission spectrometer following a micro-Dumas combustion of the filter (Fiedler and Proksch 1975). The dried filters were placed in pyrex emission tubes along with CuO powder and several small nuggets of CaO. The CuO was pre-combusted to 560°C before use and the CaO nuggets were heated to >1000°C with a torch just prior to final ampulation.

TABLE 2. Tests for recovery of the initial $^{15}N/^{14}N$ ratio in glycine following DON isolation procedure. The initial standards were ~8 atom-% ^{15}N but are reported as 100% to permit comparisons of values measured at different steps in the process during different trials. Values are listed as the percent of the initial standard atom-% ^{15}N for triplicate measurements.

Process step	Percent of initial atom-% ^{15}N ($\bar{x}$ ± SD)	Total glycine (μg N·L^{-1})
Initial standard	100	100, 1000
Filtered, oven-dried (50°C) rehydrated, spotted	88.7±1.4	1000
Freeze-dried (−30°), rehydrated, spotted	87.4±4.6	100
Full process (pathway 2, Fig. 1)	76.3±8.4	100, 1000

The tubes were evacuated to $<10^{-4}$ mTorr with a 2-in. diffusion pump/liquid N_2 trap system, sealed under vacuum, and combusted for 12−24 h at 560°C.

To determine the final percent recovery of the $DO^{15}N$, stock solutions of [^{15}N]glycine were prepared and carried through the entire sequence of steps as dissucssed above (Table 2). These tests were conducted using glycine solutions of 100 and 1000 μg N·L^{-1}. Background DON contamination in the deionized dilution water (~30 μg N·L^{-1}) was taken into account in the final percent enrichment calculations. The initial ^{15}N enrichment values were measured independently for each of the three trials using filters which had been spotted with ~10 μg N of the stock solution. The final percentage recovery was 76.3 ± 8.4 ($\bar{x}$ ± SD, Table 2). We were not able to identify a specific step in the procedure which could account for most of this decrease in the $^{15}N/^{14}N$ ratio. Approximately half of the decrease was found to occur during the zinc-reduction/alkalization/sparging/floc-filtration steps, and the remainder was associated with the evaporation/rehydration/spotting steps. Since it is unlikely that any of these procedures would selectively discriminate against ^{15}N, the decreased ratio is probably due to contamination with ambient nitrogen, thereby lowering the ^{15}N content by dilution. However, a consistent recovery value for $DO^{15}N$ standards can be used to accurately estimate final sample $DO^{15}N$ enrichments. Each experimenter should of course determine system-specific efficiency values using enough replicates to obtain the desired variance.

Although we did not test our method on marine samples, we note that it would not be possible to rehydrate the total salt residue of a 250-mL sample in a few millilitres of deionized

water. Pretreatment of the sample using ion-exchange resins to remove NaCl should prevent this problem, but tests should be conducted to ensure that DON is not removed in the process.

In conclusion, we have presented a simple and inexpensive method for the isolation and direct measurement of $DO^{15}N$. For samples initially spiked with $^{15}NH_4^+$ the removal of $DI^{15}N$ will be nearly 100%, but if $^{15}NO_3^-$ is used the removal efficiency will be >98%. The magnitude of error caused by carryover of $^{15}NO_3^-$ into the measured $DO^{15}N$ pool will of course depend on the final concentration and atom-% ^{15}N enrichments of the nitrate and organic N pools. We developed this procedure to estimate the rate of incorporation of ^{15}N from nitrate into DON in oligotrophic Castle Lake, California. Microcosm, mesocosm, and whole-lake studies of DIN cycling had not completely delineated the fate of allocthonous nitrate inputs in this N-deficient lake (e.g. Axler et al. 1980, 1982). Our finding that the ^{15}N enrichment of the DON pool in $^{15}NO_3^-$-enriched mesocosms was <1 atom-% (e.g. Table 1; a complete report is in preparation), which corresponded to <2% of the total $^{15}NO_3$ amendment, now suggests that littoral sediments (i.e. periphyton and denitrifying bacteria) are a major sink for nitrate in this lake (Axler et al. 1984; Reuter et al. 1985).

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Effects of Coal Leachates on Fish Spermatogenesis

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Cochran, R. C. 1987. Effects of coal leachates on fish spermatogenesis. Can. J. Fish. Aquat. Sci. 44: 134–139.

Coal leachates were examined for toxic effects on fish spermatogenesis. Nonbreeding mummichogs, *Fundulus heteroclitus*, were induced to enter breeding condition in the laboratory by exposure to 20°C and a photoperiod of 16L:8D for 6 wk. During this 6-wk period, mummichogs were dosed in static exposure tanks with water extracts of coal (leachates). Chi-square analysis and *Z* test of proportions revealed a significant reduction in sperm production by fish exposed to some, but not all, coal leachates. This reduction was as much as 40-fold and occurred in a dose-dependent manner. Ordinarily, nonbreeding mummichogs collected immediately after the breeding season will not enter breeding condition in response to 16L:8D and 20°C. Exposure of these photoinsensitive mummichogs to coal leachates under long photoperiod conditions for 8 wk resulted in a significant increase in sperm production. Analysis of weekly sperm production by mummichogs from six field populations, either adjacent to or 2 miles upstream from coal-fired power plants, did not reveal significant differences during the breeding season. However, this sperm production was less than 1/10 that of a mummichog field population sampled concomitantly at the Chesapeake Bay Institute. The reduced sperm levels could not be related to reproductive toxin(s) contained in coal leachate.

Les lessivats de charbon ont été testés pour leurs effets toxiques sur la spermatogénèse des poissons. Des fundulus hétéroclites (*Fundulus heteroclitus*) non reproducteurs ont été induits à passer en reproduction au laboratoire par exposition à une température de 20°C et à une photopériode de 16J:8N pendant 6 sem. Durant cette période, des extraits de charbon solubles dans l'eau (lessivats) étaient versés dans les bassins statiques des poissons. Le test du chi carré et le test *Z* ont révélé une réduction significative de la production de laitance par les poissons exposés à certaines lessivats du charbon, mais pas tous. Cette réduction pouvait atteindre 40 ordres de grandeur et elle était liée à la dose. Ordinairement, les fundulus non reproducteurs qui sont capturés immédiatement après la saison de la reproduction n'entrent pas en état de reproduction quand ils sont soumis au traitement 16J:8N à 20°C. L'exposition de ces sujets photoinsensibles aux lessivats du charbon à une photopériode prolongée pendant 8 sem a conduit à une augmentation significative de la production de laitance. L'analyse hebdomadaire de la production de laitance par des fundulus prélevés dans six populations qui vivaient dans des eaux contiguës à des centrales alimentées au charbon ou 2 milles en amont, n'a pas révélé de différence significative durant la saison de la reproduction. Cependant, cette production de laitance était moins d'un dixième de celle d'une population échantillonée au même moment à l'Institut de Chesapeake Bay. Il est impossible d'établir un rapport entre la concentration réduite de laitance et certaines toxines à effet sur la reproduction et contenues dans les lessivats de charbon.

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The use of coal as a fuel source for power plants often involves the storage of coal on the plant site. Coal stored in this manner is subject to leaching by rain or groundwater, and the leachates may seep into surface waters. Leachates from coal contain polycyclic aromatic hydrocarbons, heavy metals, and other substances (Torrey 1978; Brown 1979) which might have toxic effects on fish reproduction. Indeed, a laboratory study has shown that coal leachate can reduce sperm production by fish induced to enter a breeding condition (Cochran 1985).

The present study was undertaken to answer three questions about the toxin(s) in coal leachate which affects fish sperm production. First, is it possible to demonstrate diminished sperm production by fish in the Chesapeake Bay exposed to leachates from coal piles at power plants? Second, does all coal, regardless of the geographic origin, possess toxin(s) which can diminish fish sperm production? Lastly, what is the mechanism of action of the toxin(s) in affecting sperm production?

The mummichog, *Fundulus heteroclitus* (Teleostei, Cyprinidontidae), was used as a model species in which to study the effects of coal leachates on fish spermatogenesis for a number of reasons. First, mummichogs are ubiquitous in the Chesapeake Bay (Hildebrand and Schroeder 1928), so the field populations can be sampled near each of the coal-fired power plants located on the Bay. Second, as mummichogs remain within a range of 35 m during the breeding season (Lotrich 1975), the effect of the local environment on sperm production in a specific population can be studied. Third, mature male mummichogs are small and easily captured and maintained in the laboratory in large numbers (Joseph and Saksena 1966). Finally, the gametogenic cycle of mummichogs is short and can be manipulated in the laboratory using changes in photoperiod and water temperature (Day and Taylor 1983, 1984; Taylor

1984). Thus, the effects of coal leachate on fish spermatogenesis can be studied with mummichogs in approximately the same reproductive condition throughout the year.

Materials and Methods

Mature male mummichogs, greater than 3 cm standard length, were collected in Parrish Creek adjacent to the Johns Hopkins University's Chesapeake Bay Institute (CBI) for laboratory studies. For the field studies, 10 mummichogs were collected in baited lift nets at weekly intervals during the breeding season (April through August) 200 m from the boundary of each of three coal-fired power plants: Brandon Shores (Baltimore Harbor), Chalk Point (Patuxent River), and Morgantown (Potomac River). These collection points were approximately 500 m from the coal piles. Weekly collections were also made at each of three reference sites 2 miles upstream from the power plants at Curtis Bay, Patuxent River Park, and Pope's Creek, respectively. The mummichogs were transported to the Institute in aerated buckets of estuarine water.

Testicular Growth and Measurement of Sperm Index

Under natural conditions, mummichogs and other fish in the Chesapeake Bay breed between April and August each year (Matthews 1938). The change from nonbreeding to breeding condition involves testicular growth, as mummichog testes increase 40- to 50-fold in size. Associated with the increase in size is a dramatic increase in the testicular production of spermatozoa. Effects of leachates on fish spermatogenesis were examined during this process of testicular growth so that the physiological condition of the fish could be standardized and the maximum effect of toxins on spermatogenesis could be observed.

Nonbreeding fish were transferred from holding tanks (kept under short photoperiod [8L:16D] to 40-L tanks kept at 20°C and long photoperiod [16L:8D] to induce testicular growth. Fish were fed ad libitum on commercial flake food (Tetramin, Tetrawerke, West Germany). Such an environment induced breeding condition in 6 wk. Mummichogs, 10 in each 40-L tank, were exposed to leachates or kept in estuarine water as controls under static conditions. Estuarine water or leachates were changed weekly in each tank.

Sperm production was assessed by determining the sperm index (Cochran 1985) as follows. Mummichogs were weighed and the testes were removed and weighed. The testes were then homogenized for 3 min in 200 mL of a solution (150 m*M* NaCl and 0.05% (v/v) Triton X-100) with a Waring blendor. After homogenization, a drop of homogenate was placed on each side of a hemacytometer and condensed heads of spermatids and spermatozoa were counted under a phase contrast microscope at 400×. The total number of condensed heads per testes was multiplied by the gonosomatic index ((testes weight/body weight) × 100) to obtain the sperm index. The data were expressed as a sperm index to take into consideration the fact that larger testes contain more sperm and larger fish tend to have larger testes.

Statistical analysis of the data was performed using the *Z* test for proportions (mean values were considered significant if they differed by more than 3 standard deviations), chi-square analysis, and the Mann–Whitney–Wilcoxon test (Snedecor and Cochran 1967).

Laboratory Studies

1. Effects of coal leachates on spermatogenesis in photosensitive fish

Crushed coal samples, used by Maryland power companies to determine moisture content of freshly delivered coal, were obtained from mines in Kentucky, Pennsylvania, and western Maryland. Coal samples were also obtained from onsite piles of coal at the Brandon Shores, Wagner, and Chalk Point power plants. These latter coal samples consisted of nonuniform-sized chunks of coal, ranging from 50 cm^3 to dust.

Leachates of coal were prepared by mixing 50 kg of coal with estuarine water (1 kg/decaliter) at room temperature (20°C) in open 200-gal fiberglass tanks for 6 wk. The mixture was agitated by airstones. The pH of the leachates was adjusted to 7.8 with 1 *M* Na_2CO_3, when necessary, before being used to dose fish. Adjustment of pH had no effect on the salinity (11 ppt) of the leachates.

At the start of each experiment the sperm indices of 10 nonbreeding mummichogs, kept in holding tanks under short photoperiod [18L:16D] and low temperature [<10°C], were determined as initial controls. Nonbreeding mummichogs were then transferred from these holding tanks to 40-L tanks (10 fish per tank) containing leachates and exposed to long photoperiod [16L:8D] and 20°C for 6 wk. Associated with each experiment, one group of 10 males in estuarine water was exposed concomitantly to long photoperiod [16L:8D] and 20°C for the 6 wk as a breeding control. A second control group of 10 mummichogs in estuarine water was exposed concomitantly to short photoperiod [8L:16D] and 20°C for 6 wk as a nonbreeding control. At the end of this time, the sperm indices of all fish were determined.

2. Effects of coal leachates on spermatogenesis in photoinsensitive fish

Ordinarily, mummichogs collected during the first 12 wk following the breeding season do not exhibit testicular growth in response to photoperiodic stimulation in the laboratory (Day and Taylor 1984; Cochran 1985). Preliminary results (Cochran 1985) had shown a paradoxical "stimulatory" effect of coal leachates on sperm production by refractory fish. To obtain a better insight into the mechanism of action of leachate toxin(s), photoinsensitive mummichogs were exposed to leachates in two separate experiments. In the first experiment, leachate was made from coal from the Chalk Point coal pile. In the second experiment, fresh, crushed coal samples from a Kentucky and a Maryland mine were used to make leachates.

In each experiment the sperm index of 10 mummichogs was immediately determined as the initial control. Ten fish were placed in each 40-L tank containing estuarine water (control), or different dilutions of leachates, and kept under long photoperiod (16L:8D) at 20°C for 8 wk. In the second experiment a duplicate set of exposure and control tanks were kept under short (8L:16D) photoperiod at 20°C for 8 wk as described above. At the end of this time, the sperm indices of all fish were determined.

Results

Field Studies

At each of the collection sites, the sperm index of the field populations changed significantly over the course of the breeding season (Fig. 1A, 1B, 1C). However, at the time of each

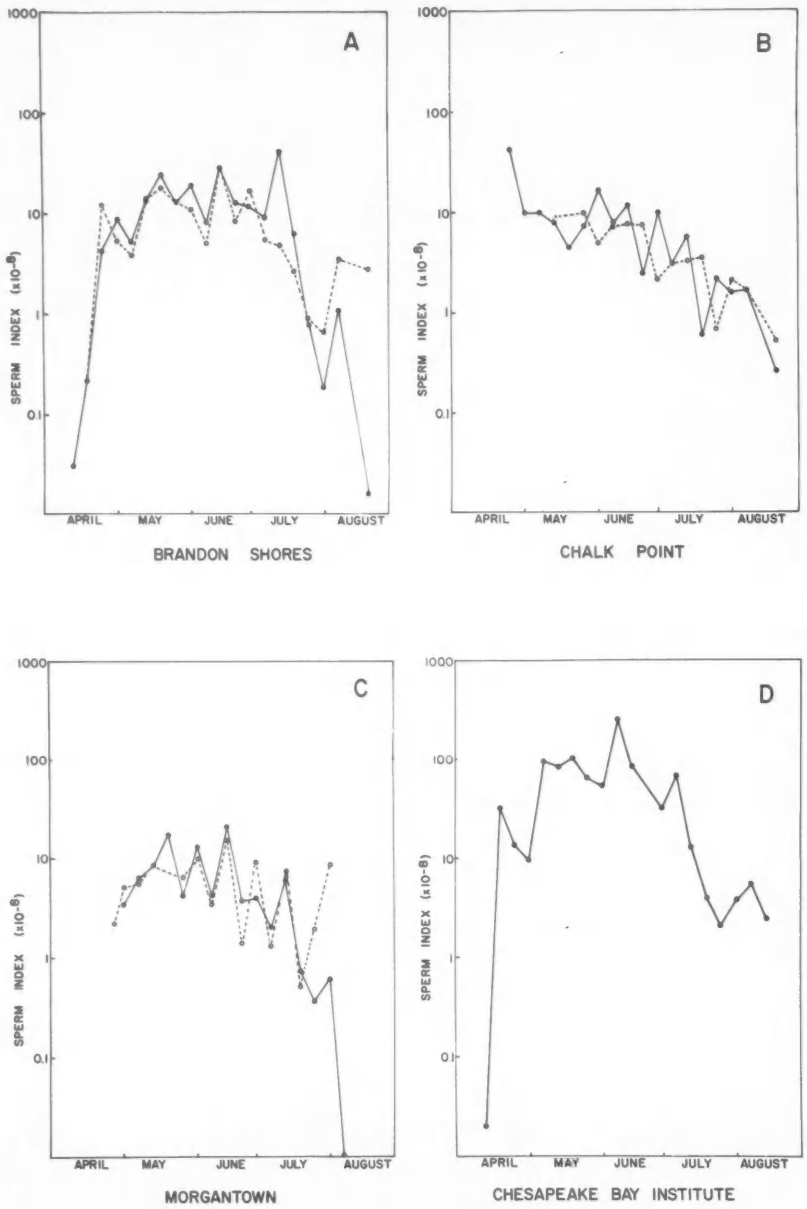

FIG. 1. Log of the sperm index in field populations of *Fundulus heteroclitus* (A) adjacent to the Brandon Shores power plant (—) or at Curtis Bay (---) 2 miles upstream, (B) adjacent to the Chalk Point power plant (—) or at a reference site (---) 2 miles upstream, (C) adjacent to the Morgantown power plant (—) or 2 miles upstream at Pope's Creek (---), and (D) adjacent to the Chesapeake Bay Institute. Each point represents the log of the mean sperm index of 10 males captured on that date.

collection during the breeding season, the sperm indices of mummichog populations at exposure sites adjacent to power plants compared with their respective reference sites were not significantly different until the end of the breeding season at the Brandon Shores and Morgantown sites. Further, the sum of the sperm indices over the entire breeding season for each of the exposure and reference populations near power plants revealed no significant differences in total sperm production.

Total sperm production during the breeding season by the field population of mummichogs sampled weekly at CBI (Fig. 1D), however, was significantly higher than those of the collection sites near power plants, or their respective reference

TABLE 1. Sperm indices (SI, mean ± SD of 10 fish) of photosensitive mummichogs determined at the initiation of an experiment and at the end of an experiment after exposure to long or short photoperiod (PP) in the laboratory in the presence or absence of stored coal leachates. *Significantly less ($P < 0.05$) than the long photoperiod control.

Leachate source	Initial (SI × 10^{-9})	Long PP (SI × 10^{-9})	Short PP (SI × 10^{-9})
Experiment 1			
Control	0.01±0.01	24.9±9.9	0.01±0.01
Chalk Point		15.2±7.8*	
Experiment 2			
Control	0.11±0.13	4.1±0.45	0.11±0.12
Wagner		3.8±2.10	
Brandon Shores		5.5±6.29	

sites. Sperm production by the CBI population in 1984 was not significantly different from sperm production by the population in 1983 (Cochran 1985).

Laboratory Studies

Photosensitive mummichogs, kept 6 wk in long photoperiod (16L:8D) and 20°C in the laboratory, usually developed a sperm index ($24.9 \pm 9.9 \times 10^{-9}$) indistinguishable from those of CBI field populations ($25.5 \pm 17.0 \times 10^{-9}$) during the height of the breeding season. Photosensitive fish, kept 6 wk in short photoperiod (8L:16D) and 20°C in the laboratory, usually had sperm indices ($0.01 \pm 0.02 \times 10^{-9}$) not significantly different from those of the initial controls ($0.01 \pm 0.01 \times 10^{-9}$) or those of nonbreeding fish ($0.02 \pm 0.01 \times 10^{-9}$) in natural populations (Tables 1,2). Because the physiological condition of the starting fish varied, however, comparisons were made only within each experimental group.

Effects of leachates

None of the mummichogs treated with lechates displayed any overt pathological symptoms. Photosensitive mummichogs, exposed to leachate under long photoperiod, developed normal breeding coloration compared with laboratory breeding controls and breeding males from field populations. The testes of these mummichogs were milky white in appearance, indicating the presence of sperm. The gonosomatic index of mummichogs treated with Pennsylvania ($3.80 \pm 0.53 \times 10^{-9}$) or Chalk Point ($3.90 \pm 0.59 \times 10^{-9}$) coal leachate was not significantly different from their breeding controls ($3.72 \pm 0.59 \times 10^{-9}$ and $3.63 \pm 0.59 \times 10^{-9}$, respectively). Histological examination of the testes with light-level microscopy revealed no obvious differences between leachate-treated fish and controls.

Photosensitive fish

The sperm index of fish (kept in long photoperiod) exposed to various dilutions of leachate from Kentucky coal was not significantly different from the breeding control. However, fish (kept in long photoperiod) exposed to Pennsylvania and Maryland coal leachates had significantly reduced sperm indices compared with their respective breeding controls (Table 1).

The effects of the Maryland coal leachate on the sperm index of fish kept in long photoperiod were concentration dependent, ranging from $0.05 \pm 0.11 \times 10^{-9}$ to $1.52 \pm 4.31 \times 10^{-9}$ compared with $2.08 \pm 2.95 \times 10^{-9}$ for long photoperiod controls. Neither nonbreeding controls nor leachate-treated fish kept in short photoperiod exhibited significant differences in sperm index compared with the initial controls (Table 1).

TABLE 2. Sperm indices (SI, mean ± SD, of 10 fish) of photosensitive mummichogs determined at the initiation of an experiment and at the end of an experiment after exposure to long or short photoperiod (PP) in the laboratory in the presence or absence of crushed, fresh coal leachates. *Significantly less ($P < 0.05$) than the long photoperiod control.

Leachate source	Initial (SI × 10^{-9})	Long PP (SI × 10^{-9})	Short PP (SI × 10^{-9})
Experiment 1			
Control	2.2±1.4	37.3±14.9	2.7±0.9
Pennsylvania		8.7±6.9*	
Experiment 2			
Control	0.01±0.03	2.08±2.95	0.018±0.036
Kentucky			
Undiluted		1.05±2.17	0.004±0.003
1:1 dilution		1.53±3.37	0.003±0.001
1:3 dilution		3.22±5.57	0.014±0.033
Maryland			
Undiluted		0.05±0.11*	0.009±0.014
1:1 dilution		0.14±0.39*	0.004±0.005
1:3 dilution		0.49±1.44*	0.006±0.005
1:7 dilution		1.52±4.31	

The sperm index of mummichogs (kept in long photoperiod) exposed to leachates made from coal taken from coal piles at the Wagner ($3.8 \pm 2.1 \times 10^{-9}$) and Brandon Shores ($5.5 \pm 6.3 \times 10^{-9}$) power plants was not significantly different from the concomitant breeding controls ($4.1 \pm 0.5 \times 10^{-9}$) (Table 1). This contrasts with the effects of leachate made from coal obtained from the coal pile at the Chalk Point plant, which did cause a significant reduction in the sperm index of fish in long photoperiod ($15.2 \pm 7.8 \times 10^{-9}$) compared with the breeding control ($24.9 \pm 9.9 \times 10^{-9}$). In both experiments the testes of breeding controls contained significantly more sperm than either the concomitant respective nonbreeding control or the respective initial control.

Photoinsensitive fish

Chi-square analysis and the Z test of proportions revealed no significant differences in sperm indices, or gonosomatic indices between photoinsensitive fish, which do not undergo testicular growth in response to photoperiodic stimulation, kept in either long or short photoperiod for 8 wk (Table 3). None of the photoinsensitive fish kept under short photoperiod and exposed to different concentrations of leachates from crushed coal from Kentucky or Maryland had significantly different sperm indices compared with the short photoperiod controls ($0.006 \pm 0.004 \times 10^{-9}$). Photoinsensitive fish kept under long photoperiod in various dilutions of lechate from crushed Kentucky coal also did not have significantly different sperm indices compared with breeding controls ($0.006 \pm 0.005 \times 10^{-9}$).

This contrasts, however, with the response of photoinsensitive fish kept under long photoperiod and exposed to different dilutions of leachate (undiluted, 1:1, 1:3, 1:7 v/v) from Chalk Point coal (Table 3). The sperm indices for these leachate-treated fish were significantly different from the concomitant long photoperiod control or short photoperiod control. In a follow-up experiment, the lowest concentration of leachate (1:3) from Maryland coal produced a significantly increased sperm index ($0.04 \pm 0.11 \times 10^{-9}$) compared with either the

TABLE 3. Sperm indices (SI) (mean ± SD of 10 fish) of photoinsensitive mummichogs at the initiation of an experiment and at the end of an experiment after exposure to long or short photoperiod (PP) in the laboratory in the presence or absence of coal leachates. *Significantly different ($P < 0.05$) from the long photoperiod control.

Leachate source	Initial ($SI \times 10^{-9}$)	Long PP ($SI \times 10^{-9}$)	Short PP ($SI \times 10^{-9}$)
Experiment 1			
Control	0.02±0.02	0.02±0.03	0.02±0.04
Chalk Point			
Undiluted		0.16±0.28	
1:1 dilution		1.05±2.46*	
1:3 dilution		2.21±4.23*	
1:7 dilution		0.10±0.29	
Experiment 2			
Control	0.004±0.003	0.006±0.005	0.006±0.004
Kentucky			
Undiluted		0.008±0.006	0.004±0.002
1:1 dilution		0.010±0.009	0.009±0.007
1:3 dilution		0.062±0.153	0.007±0.007
Maryland			
Undiluted		0.003±0.003	0.008±0.007
1:1 dilution		0.008±0.011	0.006±0.009
1:3 dilution		0.139±0.106*	0.004±0.001

breeding ($0.01 \pm 0.01 \times 10^{-9}$) or nonbreeding controls ($0.01 \pm 0.01 \times 10^{-9}$) (Table 3). As with the previous results, the increase in the sperm index was accompanied by the appearance of male breeding coloration.

Discussion

Field Studies

The first question to be addressed was whether sperm production by fish populations near power plants had been affected by leachates from coal piles. Sperm indices of male mummichogs in the six field populations sampled either adjacent to or 2 miles upstream from coal-fired power plants at each time point or over the entire breeding season were not significantly different from one another (Fig. 1A, 1B, 1C). Thus, no effect of coal leachate on mummichog sperm production could be demonstrated in field populations.

The fact that total sperm production during the breeding season by exposure and reference mummichog populations near power plants (Fig. 1A, 1B, 1C) was an order of magnitude less than that of the CBI mummichog population (Fig. 1D) over the same period could have been the result of many environmental factors. These factors might include differences in the genetics of the fish populations, nutrition, or other complex ecological interactions. Thus, the lower sperm indices may or may not be related to reproductive toxin(s) similar to those contained in coal leachates.

Laboratory Studies

Some, but not all, coal tested contained water-soluble toxins which caused significant reductions in sperm production by photosensitive fish exposed to the coal leachates (Tables 1,2). The effect on sperm production was dependent upon the concentration of the leachates.

Paradoxically, the same leachates, which diminished sperm production in photosensitive fish (Table 2), stimulated sperm production in photoinsensitive fish (Table 3). Normal breeding coloration was acquired by these leachate-exposed fish, with a significant increase in the sperm index compared with both the breeding and nonbreeding controls. The greatest enhancement of sperm production by photoinsensitive fish kept in long photoperiod was at lower concentrations of leachate. Indeed, the data suggest a reverse dose effect.

In searching for a possible mechanism of action for inhibition, a direct cytotoxic effect on the testes was deemed unlikely because of the lack of obvious histological differences between the testes of leachate-affected mummichogs and concomitant controls. The hormonal system seemed to be qualitatively intact as (1) the breeding coloration of leachate-treated photosensitive mummichogs appeared normal, (2) a normal increase in the gonosomatic index occurred in leachate-treated, photosensitive mummichogs, and (3) no breeding coloration or change in gonosomatic or sperm index occurred in leachate-exposed mummichogs (photosensitive or insensitive) kept in short photoperiod. As no measurements were made of the serum hormone levels, an inhibitory effect on the quantitative hormonal output of the brain, hypothalamus, pituitary, or testes cannot be excluded.

With regard to the stimulatory effect, studies have shown that the photoinsensitivity of seasonally breeding birds is due to a lack of steroidogenic gonadotropin secretion by the pituitary (Turek et al. 1980). Even in photosensitive birds, the testis remains responsive to gonadotropins and androgens (Turek et al. 1976; Desjardins and Turek 1977). Thus, the source of photoinsensitivity in birds lies in the brain, somewhere between exposure to the photoperiodic signal, its reception, and translation into an increased release of gonadotropin by the pituitary (Turek et al. 1980). Similar studies have not been conducted in mummichogs, but it is known that the photoreceptors involved in the photoperiodic response of mummichogs are extraretinal (Day and Taylor 1983).

The significantly increased sperm index of mummichogs kept in long photoperiod and treated with leachate from Maryland and Chalk Point coal suggests that the photoreceptors in photoinsensitive mummichogs can receive the photoperiodic signal and that the leachate affects the translation of that signal into gonadotropin release. If the stimulatory effect resulted from direct stimulation of the hypothalamus, pituitary, or testes to accelerate spermatogenesis, then sperm production should have been seen in refractory mummichogs kept in leachate under short photoperiod. Only photoinsensitive mummichogs exposed to Maryland or Chalk Point coal leachate, kept in long photoperiod, developed significant amounts of sperm and normal breeding coloration. These results are consistent with the hypothesis that the stimulatory effect arises from interference with the photoreceptor signal. However, much work remains to be done in proving both the mechanisms of action and the identity of the toxin(s).

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Regulation of Tin and Methyltin Compounds by the Green Alga *Enteromorpha* under Simulated Estuarine Conditions

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The cycling of inorganic tin and methyltin compounds was examined in the presence of the green alga *Enteromorpha* at different salinities. We monitored flasks containing live and dead *Enteromorpha* under oxic experimental conditions for 17 d and determined inorganic tin and methyltin compounds by chromatography – atomic absorption spectrometry. Addition of acidic inorganic tin to some flasks resulted in plant decay. Processes associated with healthy and decaying plant material included release of inorganic tin and methyltin compounds, their interconversions in solution, and uptake of inorganic tin. *Enteromorpha* releases inorganic tin as well as mono-, di-, and trimethyltin. Added inorganic tin was mostly removed from solution after its addition, and all three methyltin compounds were produced. The methyltin compounds cycle such that high concentrations of trimethyltin were concomitant with low mono- and dimethyltin concentrations and vice versa. We describe possible biological and chemical processes responsible for interconversions of inorganic tin and methyltin compounds in solution and the environmental significance of seaweed in the biogeochemical cycling of tin.

Les auteurs ont étudié le cycle de l'étain inorganique et des composés de méthylétain en présence de l'algue verte *Enteromorpha* cultivée à différentes salinités. Pendant 17 j, ils ont suivi l'activité dans des flacons contenant *Enteromorpha* morte ou vivante en milieu expérimental oxique; la présence d'étain inorganique et des composés de méthylétain a été déterminée par spectrométrie par absorption atomique et par chromatographie. L'apport d'étain inorganique acide dans certains flacons a entraîné la décomposition végétale. La libération d'étain inorganique et de composés de méthylétain, leur interchangeabilité en solution et l'absorption d'étain inorganique sont des processus liés aux matières végétales en santé et en décomposition. En plus de l'étain inorganique, *Enteromorpha* relâche du mono-, du di- et du triméthylétain. L'étain inorganique ajouté a été en grande partie éliminé de la solution après l'ajout, ce qui a été suivi de la production des trois composés de méthylétain. Ces derniers parcourent un cycle qui mène à des concentrations élevées de triméthylétain accompagnées de faibles concentrations de mono- et de diméthylétain et vice versa. Les auteurs décrivent les processus biologiques et chimiques qui peuvent causer l'interchangeabilité de l'étain inorganique et des composés de méthylétain en solution, et l'importance environnementale des algues dans le cycle biogéochimique de l'étain.

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Pollution by organotin compounds and methylation of inorganic tin to produce methyltin compounds of considerably enhanced toxicity (Thompson et al. 1985) make it necessary to understand their effects on the aquatic environment. Several algae are involved in biogeochemical cycles of methylarsenic compounds (Andreae and Klumpp 1979; Sanders and Windom 1980; Baker et al. 1983). Algae can also concentrate inorganic tin and methyltin compounds (Seidel et al. 1980; Ishii 1982; Wong et al. 1984) and degrade *n*-butyltin compounds (Maguire et al. 1984). However, many of these compounds are highly toxic to algae (Wong et al. 1982). Finally, live and decaying algae can release exudates that bind metal ions and decrease their toxicity (Lobban et al. 1985).

Although considerable information is available on the bioaccumulation of metal ions by algae and the complexation of metal ions by algal exudates, little is known about the impact of living and decaying algae on the formation and stability of organometallic compounds including those of methyltin. In addition, most studies on tin–algae relationships used micro-

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scopic algae and not seaweeds which can make a large contribution to estuarine biomass and can cause cycling of metals in coastal waters during the entire year. Furthermore, seaweeds contain and release potential methylating compounds (Lovelock 1975; Reed 1983; Gschwend et al. 1985).

This paper describes the influence of species of the green alga *Enteromorpha* on the formation and stability of methyltin compounds in a model system using diluted artificial seawater. *Enteromrpha* can accumulate inorganic tin and methyl- and *n*-butyltin compounds, as well as release and interconvert methyltin compounds. We discuss possible mechanisms for these processes.

Materials and Methods

Chemicals

Distilled and deionized water was used in all experiments. All chemicals unless otherwise stated were of analytical quality. The artificial seawater used (Kester et al. 1967) has an inorganic tin concentration of 0.30 ng/mL and no detectable methyltin compounds. A 1.000 mg/mL inorganic tin solution was prepared by dissolving 1.000 g of 99.9% pure 20-mesh tin metal (Alfa Ventron) in 100 mL of 12 *M* HCl and adjusting the final volume to 1 L with water. HNO_3 was Baker Ultrex quality. Aqueous solutions of 4% $NaBH_4$ were prepared from 99% $NaBH_4$ (Alfa Ventron). Reaction flasks were soaked in 30% KOH for 2 h and rinsed with water. All other glassware and filter holders were immersed in 7% HCl for 12 h and rinsed in water.

Seaweed Samples

Several of the common intertidal seaweeds *Enteromorpha flexuosa* ssp. *paradoxa*, *Enteromorpha prolifera*, *Ascophyllum nodosum*, *Fucus vesiculosus* var. *spiralis*, *Ulva lactuca*, and *Polyphonia* spp. were collected in the autumn of 1984 from the Great Bay Estuary (New Hampshire) near the Jackson Estuarine Laboratory (JEL). Samples were selected from healthy, nonreproductive portions of the plants. The samples were rinsed sequentially in Great Bay water and in artificial seawater, gently blotted dry with tissue, and immediately placed in the experimental setup described below.

Analyses

Analyses of seaweed tissues were performed by hydride generation, cryogenic trapping, and separation on 10% SP-2100 on Chromosorb W (100–120 mesh) chromatographic packing material. Detection was done in a heated quartz furnace aligned in the beam of a 503 Perkin-Elmer atomic absorption spectrometer (AAS) at 235 nm. Results are expressed as nanograms of tin per gram (wet weight). Additional details are available (Donard et al. 1986).

Three-millilitre aqueous samples from the flasks were filtered and then digested with 2 mL of 0.1 *M* NaOH overnight at room temperature. Samples were analyzed after acidification to pH 2 with HNO_3 and adding 1.5 mL of 4% $NaBH_4$. The resulting hydrides were trapped at −196°C (liquid nitrogen) and separately eluted from the column by warming. Elution times were as follows: SnH_4, 0.44 min; $MeSnH_3$, 1.59 min; Me_2SnH_2, 2.38 min; Me_3SnH, 3.34 min; and Me_4Sn, 4.12 min. For calibration solutions, relative standard deviation (RSD) is 10% and the detection limit (3σ of background) is 5 pg/mL. Because of the heterogeneity of the seaweeds, RSD varied between 3 and 100% for replicate analyses of solutions containing them.

Digestion of plant tissues was done by immersing 0.2-g samples of blotted, dry tissue in liquid nitrogen, crushing with a mortar and pestle, and homogenizing with 5 mL of water for 10 min in a Teflon Virtis tissue grinder. The mixture was digested with 5 mL of 1 *M* NaOH for 48 h. All samples were reacidified to pH 2 with 5 *M* HNO_3 before analysis.

Determination of Me_4Sn in seaweed tissues was done by purging digested samples using our AAS procedure (Donard et. al, 1986), except for the absence of $NaBH_4$. Detection of Me_4Sn in headspaces of flasks was done by injection of 0.5 mL of headspace into a Varian model 3700 gas chromatograph with a flame ionization detector. A 1.8-m (6-mm inside diameter) column packed with 10% SP-2100 on Chromosorb G (100–120 mesh) was used at 50°C. The N_2 carrier gas flow was 30 mL/min and the retention time was 3.0 min.

Experimental Setup

Each 1-L Pyrex round-bottom flask had an air inlet frit at the bottom and a neck containing a three-way stopcock. The stopcock outlets could be sealed by silicone septa to allow sampling of headspace. Compressed "air" (an oxygen–nitrogen mixture) was used to keep the flasks well mixed. The 50 mL/min air flow through Tygon tubing was monitored with a Gilmont flowmeter.

The flasks were filled with 1 L of diluted artificial seawater. Four flasks each at salinities of 5, 17.5, and 30 ppt (grams per kilogram) were used. Salinities were measured by an American Optic refractometer. No nutrients were added to the flasks. Then, 5 g of cleaned and blotted-dry *Enteromorpha* sp. (*E. flexuosa* ssp. *paradoxa* and *E. prolifera*) was added to each flask and gently stirred to unfold them. The seaweeds were allowed to equilibrate 4 d. After the equilibration period, an acidic inorganic tin solution was added to 10 flasks for a final Sn concentration of 5 μg/mL. No inorganic tin was added to two control flasks (salinity 17.5 ppt). Thus, the experimental design comprised four tin-amended replicates at salinity 30 and 5 ppt and two replicates at salinity 17.5 ppt and two control replicates at salinity 17.5 ppt. The acidic inorganic tin solution decreased the pH to 2. The result of the low pH was that the seaweeds rapidly lost gases, as shown by bubbles on their surface, and their color quickly changed from green to yellow–brown. The pH was immediately readjusted to 8 with small amounts of 10 *M* NaOH. During the entire experiment the seaweeds were subjected to natural photoperiods at room temperature (20–26°C). Concentration changes in inorganic tin and methyltin compounds were monitored at days 4 (before addition of tin), 7, 12, and 17. The air flow was stopped and the flasks were sealed 24 h before noontime sampling of headspace (~30 mL) and removal of a 3-mL aliquot from each flask using disposable plastic syringes. The aliquots were immediately filtered with a 0.45-μm Millipore filter and analyzed as described above. The pH values were measured after sampling.

Results

Concentrations of methyl- and *n*-butyltin compounds in several seaweeds (Table 1) clearly indicate that they concentrate alkyltin compounds relative to surrounding estuarine waters (Donard et al. 1986). Accumulation factors based on

TABLE 1. Concentration (ng/g wet weight) of inorganic tin and alkyltin compounds in several seaweeds (nd = not detected). Values in parentheses are percent of total tin. Dry weight/wet weight was 0.09.

	Enteromorpha	*Ascophyllum*	*Fucus*	*Polysiphonia*
Inorg. Sn	395 (72)	217 (80)	360 (75)	183 (73)
$MeSn^{3+}$	47 (9)	30 (11)	71 (15)	45 (18)
Me_2Sn^{2+}	52 (9)	13 (5)	47 (10)	22 (9)
Me_3Sn^{+}	1 (<1)	nd	3 (<1)	nd
Me_4Sn	nd	6 (2)	nd	nd
$(n\text{-}Bu)Sn^{3+}$	6 (1)	6 (2)	2 (<1)	nd
$(n\text{-}Bu)_3Sn^{+}$	50 (9)	nd	nd	nd

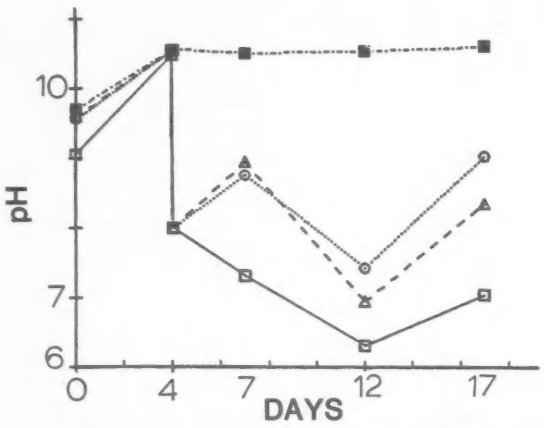

FIG. 1. Changes in pH after a 4-d equilibration period of macroalgae with no addition of tin for control flask salinity 17.5 ppt (■), and treatment flasks: 5 ppt (□), 17.5 ppt (○), and 30 ppt (△).

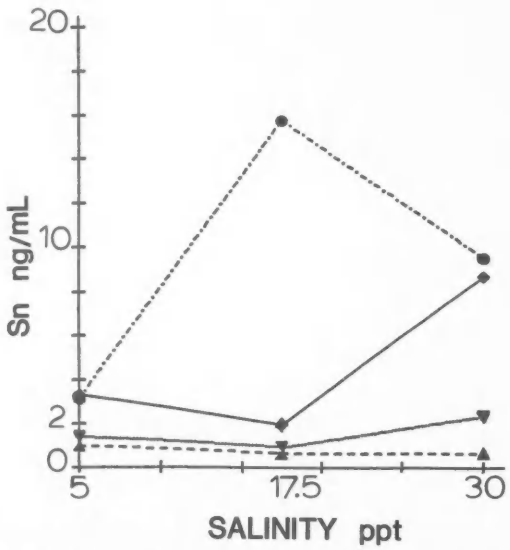

FIG. 2. Release of inorganic tin and methyltin compounds after a 4-d equilibration period of macroalgae with no addition of tin. ◆, inorganic tin; ▼, monomethyltin; ▲, dimethyltin; ●, trimethyltin.

total tin content and comparison with average total tin concentration in the Great Bay Estuary during autumn are about 10^3, comparable with those observed for other metals (Lobban et al. 1985).

The pH of treatment flasks decreased between days 7 and 12 and then increased to day 17 (Fig. 1). The pattern of pH fluctuation in treatment flasks coincided with observed metabolic plant activity: rapid oxygen release until day 4, reduced growth when plants died by day 7, and regrowth of an assemblage of epiphytes, unicellular green algae, and bacteria as shown by microscopic examination. The control flasks, which have no added tin, showed an approximate pH of 10.3 between days 4 and 17. Samples at salinity 5 ppt had lower pH values than those at higher salinities, presumably due to lower buffering capacity.

Solutions containing healthy seaweed show changes in inorganic tin and methyltin concentrations at different salinities with no addition of inorganic tin (Fig. 2). The concentration of tin compounds on day 4 was generally higher at high than at low salinity. The general order of concentration of methyltin compounds was $Me_3Sn^{+} \gg MeSn^{3+} \approx Me_2Sn^{2+}$. Headspace analysis by GC did not reveal Me_4Sn due to interferences from volatile compounds in the samples.

Solutions of decaying macroalgae show temporal changes in concentrations of tin species with changes in salinities (Fig. 3, 4, and 5). Figure 3 describes concentration changes for inorganic tin, and Fig. 5 shows them for Me_3Sn^{+}. Figure 4 describes the sum of $MeSn^{3+}$ and Me_2Sn^{2+} concentrations. In all experiments the Me_2Sn^{2+} concentration was less than 25% of the total, but the concentrations of both ions increased or decreased together. Figure 3 demonstrates that 3 d after the initial spiking with inorganic tin its concentration decreased to about 10% of the original level and remained at a low level. The final inorganic tin concentration was highest at the lowest salinity. The concentrations of $MeSn^{3+}$ plus Me_2Sn^{2+} and those of Me_3Sn^{+} varied periodically (Fig. 4 and 5). Furthermore, when the concentrations of Me_3Sn^{+} increased, those of the other two methyltin species decreased. Healthy and decaying seaweeds generally showed similar behavior in the cycling of inorganic tin and methyltin compounds. An exception was day 12 during which Me_3Sn^{+} at salinity 17.5 ppt had a 56 ng/mL concentration in flasks containing healthy algae and only a 15 ng/mL concentration in flasks containing decaying algae.

The total methyl group concentration as nanomoles per millilitre decreased from day 4 to 7, increased from day 7 to 12, and decreased from day 12 to 17 (Fig. 6). On day 17 the control flask value was very low compared with those of the treatment flasks. The values shown exclude Me_4Sn because it vaporizes from the aerated flasks.

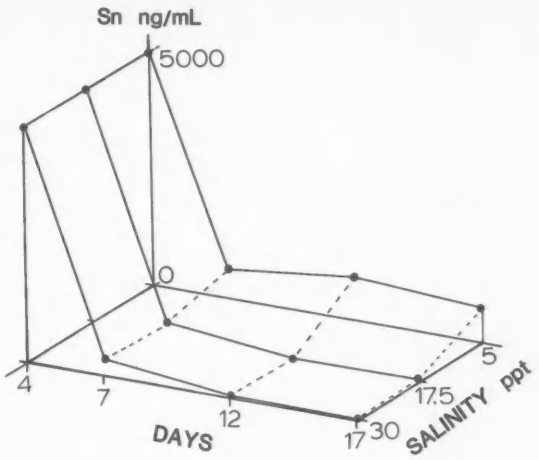

FIG. 3. Removal of inorganic tin after a 4-d equilibration period as functions of time and salinity.

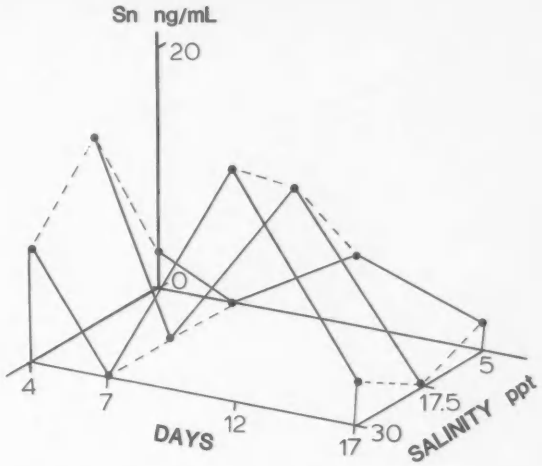

FIG. 5. Cycling of trimethyltin as functions of time and salinity.

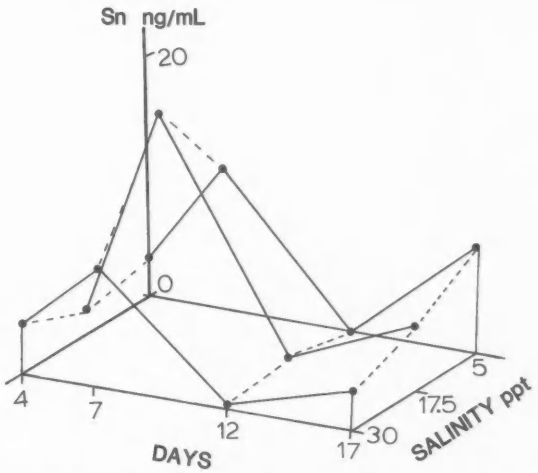

FIG. 4. Cycling of sum of monomethyltin and dimethyltin concentrations as functions of time and salinity. Dimethyltin is always a minor component of the sum.

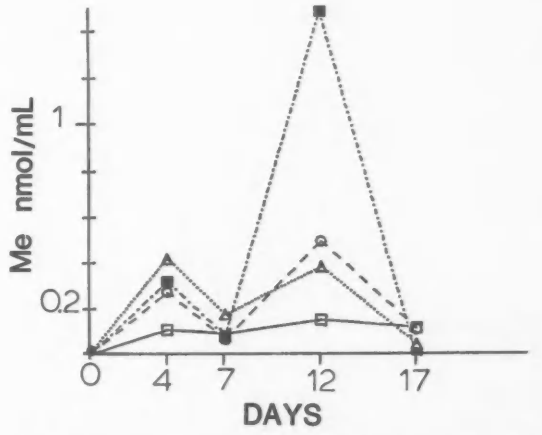

FIG. 6. Solution methyl group content for control flask, salinity 17.5 ppt (■), and treatment flasks: 5 ppt (□), 17.5 ppt (○), and 30 ppt (△).

Discussion

Seaweeds from the Great Bay Estuary (Table 1) accumulate inorganic tin and alkyltin compounds from surrounding waters (Donard et al. 1986). Seidel et al. (1980) and Ishii (1982) made similar observations with seaweeds from several California bays. The high concentrations of tin compounds associated with seaweeds might be attributable to epiphytic bacteria which have a symbiotic relationship with the algae (Cundell et al. 1977; Kong and Chan 1979; Provasoli and Pinter 1980). The bacteria can contribute up to 50% of the total metal concentration of freshwater algae (Patrick and Loutit 1977). Blair et al. (1982) observed that bacterial isolates accumulate high concentrations of *n*-butyltin compounds and emphasized the importance of bacterial colonies to the total quantity of alkyltin compounds associated with the algae. Differences in results during replicate experiments might be caused by different types of bacteria on the plant fragments used (Cundell et al. 1977; Sieburth and Tootle 1981) or differences in the algae themselves. In our discussion we will use the term "seaweed" to include the macroalga and its associated epiphytic microalga and bacteria. Variations in the seaweed assemblage influence concentrations of methyltin compounds (Fig. 2) and might explain why measured concentrations were higher than those naturally occurring in the plants (Table 1).

The pH values in control flasks were relatively constant throughout the experimental period (Fig. 1). The initial increase in pH was associated with photosynthetic activity, i.e. removal of CO_2 gas and bicarbonate ions from solution (Lobban et al. 1985). The nearly constant pH of 10.3 indicates that all CO_2 and most of the HCO_3^- was taken up during the day by plant photosynthesis. Thus, at the time of sampling the reference flasks were effectively carbon limited and chemical processes were in equilibrium. Evidence for the influence of

algal photosynthetic activity on pH due to biological activity was also apparent in the treatment flasks were minimal plant activity remained at higher salinities from day 4 to 7 and where epiphytic algae began to regrow after day 12.

Our results at all salinities tested indicate that seaweeds released inorganic tin and methyltin compounds during a 4-d equilibration period in the absence of added tin (Fig. 2). Release of $MeSn^{3+}$ and Me_2Sn^{2+} was relatively unaffected by changes in salinity, but release of inorganic tin and Me_3Sn^+ generally increased with salinity. The ability of seaweed to synthesize and release organic exudates is well documented (Carlson and Mayer 1983; Pregnall 1983), but little is known about the release of metals. However, the brown alga *Ascophyllum nodosum* releases zinc, cadmium, lead, and mercury (Eide et al. 1980), and freshwater algae release lead into a lead-free medium (Everard and Denny 1985).

Several processes can explain the release of tin compounds. First is simple desorption from the surface of the plant material:

$$(1)\quad S\cdot M \longrightarrow S + M_{(aq)}$$

where S is a plant surface and M is a metal ion, and the second is desorption due to competition of complexing exudates with the surface for tin compounds:

$$(2)\quad S\cdot M + L_{(aq)} \longrightarrow S + ML_{(aq)}$$

where *L* is a ligand. The first process, simple desorption, is likely, since algal samples were from areas having high concentrations of methyltin compounds (Donard et al. 1986), and placement of the algae in artificial seawater favors desorption. In addition, Donard and Weber (1985) showed that higher salinities decreased adsorption of Me_3Sn^+ on model environmental particulate matter. The competitive desorption process of complexation by exudates also agrees with our results. During the 4-d equilibrium phase, exudates released by the seaweed can complex the tin compounds, thus aiding their removal from the algal surface and increasing their concentration in the solution phase. Our analytical procedure does not distinguish "free" and complexed tin compounds. Wong et al. (1984) and Bond et al. (1985) observed similar effects after addition of EDTA to solutions containing live or dead algae. Similarly desorption of $(n\text{-}Bu)_3Sn^+$ from bacterial cells occurs on addition of EDTA (Blair et al. 1982). The observed processes were rapid, suggesting desorption from the seaweed surface as opposed to possible slower permeation of tin compounds through cell membranes.

Surface processes might also account for the 3-d decrease in inorganic tin concentration in solution after addition of inorganic tin (Fig. 3). These results agree with those of Wong et al. (1984), who observed rapid tin removal followed by a slower process. It is puzzling, however, that solution inorganic tin concentrations were always higher at low salinities. The usual situation in model systems is the opposite (Donard and Weber 1985). The process is obviously not a simple adsorption phenomenon.

After injection of inorganic tin on day 4, the three methyltin compounds cycled. That is, when Me_3Sn^+ concentration was low on days 7 and 17 (Fig. 5), the sum of Me_2Sn^{2+} and $MeSn^{3+}$ concentrations was high (Fig. 4). The opposite pattern occurred on day 12. The decrease in Me_3Sn^+ concentration also occurred in a separate experiment in which 100 μg of it was added to a flask containing 1 L of artificial seawater (salinity 35 ppt) and 1 g of live *Enteromorpha* spp. The result was that within 7 d, 80% of the Me_3Sn^+ disappeared from solution. Several mechanisms might account for the decrease of Me_3Sn^+ from the solution phase: (1) readsorption, (2) photolytic decomposition, and (3) a combination of biological and chemical processes. The first two processes are unlikely to be major ones. We rule out readsorption for Me_3Sn^+ because it is weakly adsorbed (Donard and Weber 1985). Photolysis is unlikely because Pyrex glass does not transmit sufficiently short wavelengths for photolysis of methyltin compounds (Blunden 1983). In addition, Blunden observed Me_2Sn^{2+} and hydrated SnO_2 as products, but we observed Me_2Sn^{2+} only as a minor methyltin product. However, we occasionally observed a transient white solid, which might be hydrated SnO_2, on the algal surface. A combined chemical–biological process could explain the inverse relationship between the concentrations of Me_3Sn^+ and of Me_2Sn^{2+} plus $MeSn^{3+}$ and the cycling of these compounds (Fig. 4 and 5). Simple chemical mechanisms cannot account for the cycling process. The importance of the biological contribution to the observed phenomena becomes clear when comparing Fig. 1 with Fig. 5 (or Fig. 4). When the pH, and concomitantly seaweed photosynthetic activity, increased, the concentration of Me_3Sn^+ decreased, particularly on days 12 and 17. We propose explanations for both decreasing and increasing Me_3Sn^+ concentrations on the basis of biological and chemical processes.

Several of our observations suggest that methylation occurs in the reaction flasks. It is important to recall that the seaweeds originally contained essentially no Me_3Sn^+ (Table 1), but that it appeared in solutions of all samples. A buildup of Me_3Sn^+ between days 7 and 12 (Fig. 5) and a significantly higher concentration of methyl groups in solution (Fig. 6) occurred simultaneously. The process occurred with decreasing pH (Fig. 1) and decreasing algal health. We believe that these observations are due to release of a carbocation (CH_3^+) donor by decaying algae. Two possible released compounds are iodomethane (Lovelock 1975) and 3-(dimethylsulfonio)propionate (DMSP) (Dickson et al. 1980; Reed 1983). Because iodomethane is very volatile, it is unlikely to be an important factor in aerated solutions, and hence, DMSP is a more likely candidate. Concentrations of methyltin compounds generally increased at higher salinities (Fig. 2 and 6), and inorganic tin concentration was lowest at high salinity (Fig. 3), in agreement with the release of methyl donors to the solution. Carbocation donors react with tin(II), but not tin(IV), compounds (Rapsomanikis and Weber 1985). We do not know how tin(II) compounds form in aerated solutions, but we suggest two possible answers to this perplexing question. First, biological exudates called siderophores are capable of complexing iron(III), reducing it to iron(II), and releasing it (Hider 1984). Second, algae are known to methylate arsenic compounds by consecutive methylation–reduction processes (Andreae and Klumpp 1979; Baker et al. 1983; Sanders and Windom 1980). Such processes might be the explanation for methylation of tin.

Trimethyltin is very toxic to aquatic life (Thompson et al. 1985) and seaweeds might detoxify it by decreasing its concentration. We hypothesize that exudates from seaweeds during times of higher biological activity (Fig. 1) decrease the concentration of Me_3Sn^+ by redistribution reactions:

$$(3)\quad 4Me_3Sn^+ \longrightarrow 2Me_2Sn^{2+} + 2Me_4Sn$$

$$(4)\quad 2Me_2Sn^{2+} \longrightarrow MeSn^{3+} + Me_3Sn^+$$

$$(5)\quad 3Me_3Sn^+ \longrightarrow MeSn^{3+} + 2Me_4Sn.$$

In agreement with our idea, certain ligands such as tropolone (Craig and Rapsomanikis 1983) or sulfide ion (Craig and Rapsomanikis 1982) induce redistribution of Me_3Sn^+ (eq. 3). Redistribution of Me_2Sn^{2+} (eq. 4) might explain why its concentration is so low. Lower methyltin group concentrations on days 7 and 17 could have occurred because Me_4Sn was purged from solution by air flow. Overall eq. 5 agrees with loss of Me_3Sn^+, formation of $MeSn^{3+}$, and removal of methyl groups via volatility of Me_4Sn.

The biogeochemical cycling of tin in the marine environment is poorly understood, with most studies of tin being primarily descriptive (Seidel et al. 1980; Froelich et al. 1985). Our studies simulated estuarine processes in the presence of fluctuating salinity and allowed observation of seaweeds in model systems. However, these model studies, together with field observation of estuarine tin levels (Donard et al. 1986) and concentrations of tin compounds in various marine algae (Table 1), strongly suggest the importance of marine plants to tin cycling in estuarine and coastal waters. Living marine algae are effective in immobolizing tin from seawater and regulating the formation and degradation of toxic methyltin compounds. Dead and decaying algae are also important in biogeochemical cycles of tin by accumulating inorganic tin and organotin compounds, releasing them, and ultimately removing tin from the estuary to the atmosphere by formation of Me_4Sn. Tin might also be removed from estuarine environments during the export of seaweeds and plant detritus. The research presented here demonstrates the potential importance of marine plants in the cycling of tin in coastal environments and establishes a background for future studies.

Acknowledgements

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Decomposition of Seston in the Hyolimnion

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Cornett, R. J., and F. H. Rigler. 1987. Decomposition of seston in the hypolimnion. Can. J. Fish. Aquat. Sci. 44: 146–151.

In 12 lakes a significant fraction of the hypolimnetic oxygen deficit was produced by the respiration of seston in the hypolimnetic water column. Mean summer seston respiration rates ranged between 4 and 80 mg $O_2 \cdot m^{-3} \cdot d^{-1}$. Rates of seston respiration were proportional to the in situ water temperature and to the concentration of Chlorophyll *a*. The amount of oxygen consumed in the water column and the fraction of the total oxygen deficit produced by sestonic respiration were correlated with the amount of phosphorus sedimented from the epilimnion. Fifteen to 66% of the total oxygen consumption occurred in the water column of the hypolimnion. Seston respiration was a larger proportion of the total respiration in the hypolimnion of lakes with a thick hypolimnion than in lakes with a shallow hypolimnetic water column.

Dans 12 lacs, le déficit en oxygène de l'hypolimnion est dû en grande partie à la respiration du seston peuplant cette zone. Les taux moyens de respiration du seston en été ont varié entre 4 et 80 $mg \cdot m^{-3} \cdot j^{-1}$ de O_2. Les taux de respiration du seston sont proportionnels à la température de l'eau *in situ* et à la concentration de chlorophylle *a*. La quantité d'oxygène consommée dans la colonne d'eau et la fraction du déficit total en oxygène lié à la respiration du seston sont en corrélation avec la quantité de phosphore de l'épilimnion qui s'est sédimenté. De 15 à 66 % de la consommation totale en oxygène a eu lieu dans l'hypolimnion. La respiration du seston représente une plus grande partie de la respiration totale dans l'hypolimnion de lacs où ce dernier forme une épaisse couche que dans les lacs où cette couche est moins épaisse.

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In the hypolimnion of lakes, the decomposition of organic matter in the water and in the bottom sediments consumes oxygen causing oxygen concentrations to decrease during thermal stratification (Birge and Juday 1911; Thienemann 1926; Hutchinson 1938, 1957). A paucity of direct measurements of water and sediment respiration has hindered the development of models of oxygen depletion and organic carbon flow through lakes. There are simultaneous measurements of sediment and water oxygen consumption in only one lake (Linsey and Lasenby 1985), few measures of sediment-oxygen consumption and total hypolimnetic oxygen depletion (Lund et al. 1963; Hargrave 1971; Lasenby 1975; Burns 1976; Rich 1979), and measurements of respiration by seston in the water in only one lake (Linsey and Lasenby 1985).

In this paper we present results of direct measures of aerobic respiration by seston in the hypolimnetic waters of 12 lakes. We have used these measurements to examine the distribution of sestonic respiration in the hypolimnion, to determine the percentage of the total rate of oxygen depletion that is contributed by respiration in the water, to test for a relation between seston respiration and the productivity of a lake's surface waters, to test the hypothesis that sestonic respiration is a larger fraction of the total amount of oxygen depletion in deep lakes (Charlton 1980; Cornett and Rigler 1980; Charlton and Rao 1983), and to compare these measures with the conflicting results produced by earlier studies and hypotheses derived from empirical models based upon the areal hypolimnetic oxygen deficit model (Thienemann 1926, 1928; Alsterberg 1927; Strom 1931; Hutchinson 1938, 1957; Cornett and Rigler 1979, 1980; Charlton 1980; Charlton and Rao 1983; Chapra and Dobson 1979; Walker 1979).

Methods

During 1979 and 1980, temperatures, hypolimnetic oxygen concentrations, and rates of respiration in the water column were measured in 12 lakes located in Quebec, Canada (approximately 45°16′N, 72°9′W; see Cornett and Rigler 1984). During thermal stratification, a station located over the deepest location in the lake was sampled 6–10 times at approximately weekly or biweekly intervals. Samples collected from three to five depths within the hypolimnion were used to estimate the concentrations of chlorophyll *a* including phaeophytins (method of Holm-Hansen 1978) and the amounts of respiration occurring in the water column. Respiration by the suspended particulate matter in the water column was measured by concentrating particles (<250 μm) in 250- or 500-mL water samples onto 47-mm, 0.45-μm filters (method of Cornett and Rigler 1984, 1986). The filters were placed in each of two

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60-mL glass bottles filled with hypolimnetic water collected from the same depth stratum. NaN_3 added to two bottles stopped respiration and served as a control to eliminate abiotic influences during the incubation. Respiration rates were determined by measuring the changes in oxygen concentration of samples and controls incubated for 1 d (about 24 h). Duplicate measurements were made at each depth in the dark at in situ temperatures. For each stratum the variance-weighted average rate of suspended particulate respiration (SPR, milligrams per cubic metre per day) was calculated for the same period as oxygen concentrations in the lake were measured. Pooling data from more than 400 duplicate experiments, the average coefficient of variation was 24% when 250-mL samples were filtered and 19% when 500-mL samples were filtered. All results were expressed as milligrams of oxygen respired since the respiratory quotient, and immediate chemical oxygen demand were not measured (Rich 1979).

Oxygen concentrations were measured using the Winkler technique (Carpenter 1965) in replicate water samples collected on each date in a vertical depth series. Eight to 18 depths were sampled in each lake. The rate of oxygen depletion at each depth and the total volume-weighted rate of oxygen depletion within the hypolimnion were calculated by regressing the measured volume-weighted average concentration of oxygen against the appropriate sampling day (Lasenby 1975).

Rates of oxygen depletion within the entire hypolimnion and within each stratum were corrected for the net amount of oxygen transported into the stratum by turbulent mixing to give the corrected net rate of oxygen respiration (NR, milligrams per cubic metre per day). The rates of vertical oxygen transport were calculated using the method of Powell and Jassby (1974) by multiplying the vertical eddy diffusivity coefficients — determined from the heat budget — times the measured gradient in oxygen concentration (see Cornett 1982; R. J. Cornett and F. H. Rigler, unpubl. data). The rate of oxygen consumption by the hypolimnetic sediments (SOD, milligrams per cubic metre per day) was calculated as the difference between NR and SPR and expressed per square metre of sediment area by multiplying the difference by the mean depth of the hypolimnion.

Results and Discussion

Distribution of SPR in the Hypolimnion

Rates of respiration were measured in the water column of seven lakes during 1979 and 12 lakes during 1980. In individual hypolimnetic strata, SPR covered a broad range: from 4 to 80 $mg \cdot m^{-3} \cdot d^{-1}$. In six lakes there were no systematic differences between rates of respiration in the different strata (e.g. Fig. 1a). In two lakes the highest SPR values were measured near the upper boundary of the hypolimnion (Fig. 1b). Higher NR values were also measured in the same strata of these lakes. Birge and Juday (1911) hypothesized that increased oxygen uptake resulted from increased rates of microbial decomposition. They believed that particulate matter collected in the upper hypolimnion and lower metalimnion because the strong density gradient decreased the settling velocity of sedimenting particles. Shapiro (1959) suggested an alternative hypothesis. He argued that the increased rates of oxygen depletion in these upper strata in Lake Washington could be accounted for solely by the respiration of large numbers of copepods that congregated in the metalimnion of the lake. However, his calculations

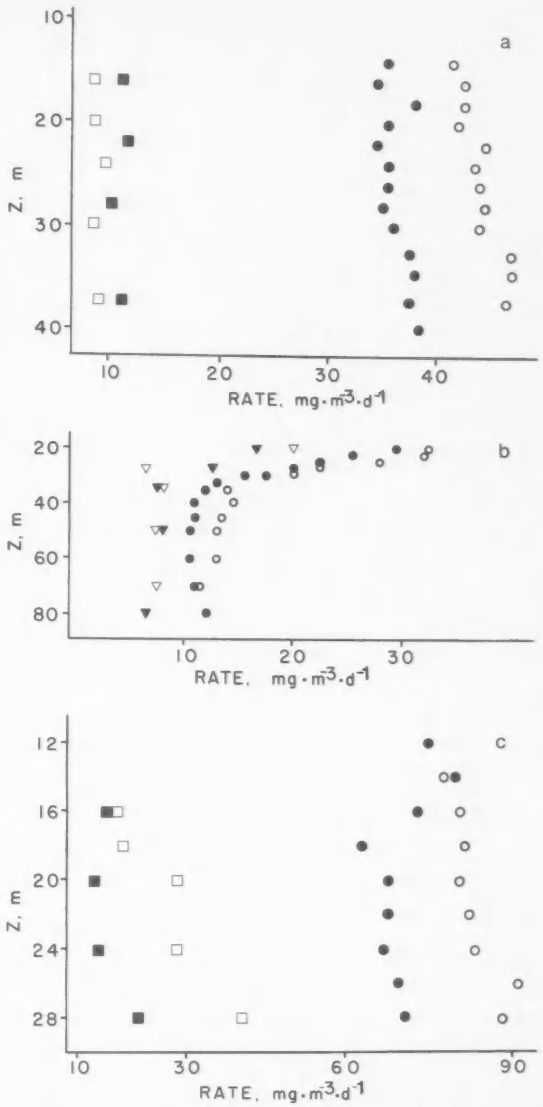

FIG. 1. Average summer net respiration (NR, circles) and suspended particulate respiration (SPR, squares or triangles) measured during 1979 (solid symbols) and 1980 (open symbols) plotted against the depth of the stratum below the lake surface (Z): (a) Lake Brompton; (b) central basin of Lake Memphremagog; (c) north basin of Lake Memphremagog.

only accounted for an average of 68% (±10%) of the respiration in the strata. Measures made in our study suggest that approximately 55% of the respiration in the strata at the top of the hypolimnion can be attributed to oxygen consumption by planktonic organisms <250 μm. These SPR values were much higher than those measured deeper in the hypolimnion (Fig. 1b). Similar results were obtained by Linsey and Lasenby (1985).

In several shallow lakes, SPR values were highest in the strata near the maximum depth of the lake (Fig. 1c). The

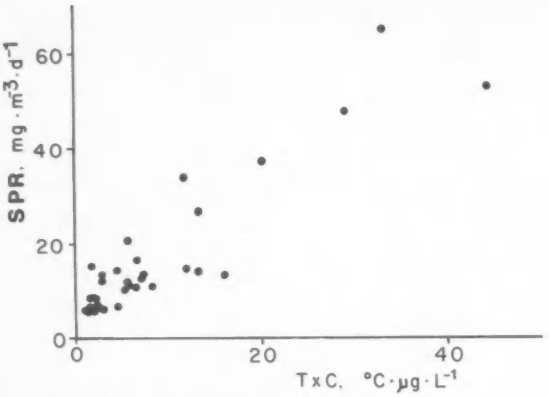

FIG. 2. Correlation between SPR values and the product of the concentation of chlorophyll *a* (*C*) multiplied by the average summer temperature (T) of the stratum.

observed increase in SPR in strata adjacent to the lake bottom is consistent with the studies of Kusnetzow and Karsinkin (1931) (cited by Hutchinson 1957), Collins (1963), and Rudd and Hamilton (1973) who observed large numbers of bacteria and higher ATP concentrations in the thermocline and in the deepest strata of the hypolimnion. Increased SPR values near the bottom were not measured in every lake. In many of the deeper lakes, the strata immediately above the bottom were not sampled. Therefore, the different patterns of SPR measured in the lakes either reflect real differences between lakes or the lack of sufficient sampling near the maximum depth. More samples were not collected near the bottom because these deep strata constitute a small fraction of the total volume of the hypolimnion. In all lakes studied, the unsampled deepest strata were less than 10% of the total hypolimnetic volume.

Relationship between Productivity and SPR

Hutchinson (1938), Cornett and Rigler (1979, 1980), Charlton (1980), and Charlton and Rao (1983) all postulated that SPR was proportional to the amount of organic matter sedimented into the hypolimnion. We tested this hypothesis in two ways: by comparing the areal rates of SPR with estimates of annual phosphorus sedimentation and with measures of chlorophyll *a* in the hypolimnion.

During 1979 the hypothesis that SPR was correlated with the concentration of organic matter was tested by measuring the amount of organic matter in the water samples used to determine SPR. Chlorophyll *a* (uncorrected for phaeophytins) was chosen as a measure of biomass because it measured the amount of autochthonous organic matter of recent origin present in the water. Testing this hypothesis was complicated by variations in hypolimnetic temperature from 4 to 12°C. Therefore, the hypothesis was tested with the multiple regression model

$$(1)\quad \mathrm{SPR} = b_0 + b_1T + b_2C + b_3T{\cdot}C$$

where C is the average summer concentration of chlorophyll *a* and T is the average summer temperature of the stratum. The regression with the smallest residual mean square was ($p < 0.001$, $R^2 = 0.62$)

$$(2)\quad \mathrm{SPR} = 5.17 + 1.20T{\cdot}C.$$

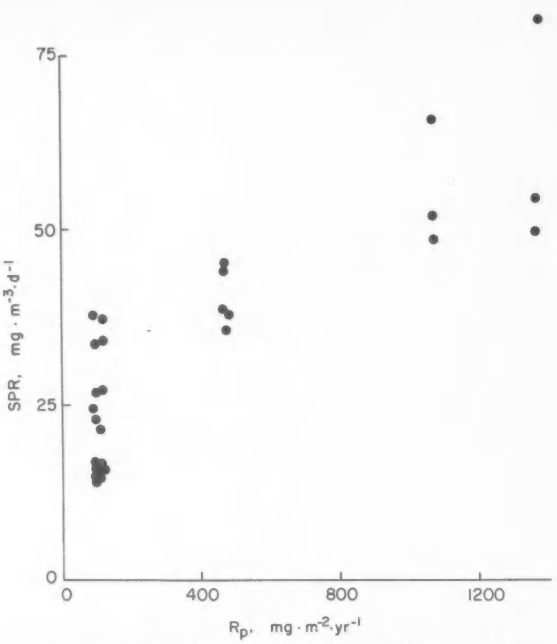

FIG. 3. Suspended particulate respiration rates (SPR) in each stratum of lakes with $Z_H > 2.5$ m and $Z_H < 4.0$ m plotted against the estimated annual phosphorus sedimentation rate (R_p).

The interaction term explained more variation in SPR than regressions with either the chlorophyll or temperature terms used alone or together (Fig. 2). Charlton and Rao (1983) also predicted that SPR was correlated with the amount of particulate organic matter input into and temperature of the hypolimnion.

Since the concentration of phosphorus is a fixed fraction of organic matter (Redfield et al. 1963), SPR should be correlated with R_p in lakes of similar depth. Areal phosphorus sedimentation (R_p, milligrams of phosphorus per square metre per year) was estimated using the mass balance model developed by Dillon and Rigler (1974) (method of Cornett and Rigler 1979). In one group of lakes with similar hypolimnetic thickness (2.5–4.0 m) but varying values of R_p (100–1360 mg $P{\cdot}m^{-2}{\cdot}yr^{-1}$), our data provide only weak support for this hypothesis (Fig. 3) ($\tau = 0.8$, $p < 0.05$) In deeper lakes ($Z_H >$ 4.0 m), SPR also appears to be correlated with Rp (Table 1). However, there were insufficient numbers of lakes with similar values of Z_H to do rigorous statistical tests.

Comparison of SPR, SOD, and NR

Volume-weighted average SPR values within the hypolimnion ranged between 6 and 65 $mg{\cdot}m^{-3}{\cdot}d^{-1}$ and NR values ranged from 12 to 216 $mg{\cdot}m^{-3}{\cdot}d^{-1}$ (Table 1). Higher SPR and NR values were measured in the shallow, more eutrophic lakes. These rates were a very significant fraction of the total amount of oxygen consumed within the hypolimnion. Even in shallow oligotrophic lakes with a high ratio of sediment area to water volume, SPR was responsible for 15–45% of NR. In the deepest lakes more than 50% of the total oxygen consumption occurred in the water column (Table 1).

Cornett and Rigler (1979, 1980) and Charlton (1980) argued

TABLE 1. Summary of the volume-weighted, mean summer rates of net respiration (NR), suspended particulate respiration (SPR), mean thickness of the hypolimnia (Z_H), and sedimentation of phosphorus (R_p) in the 12 study lakes. Errors are 1 standard error of the mean calculated by propagating errors in quadrature. Central, North, and Fitch are three separate basins of Lake Memphremagog (M). More detailed information concerning the location and characteristics of these lakes is given in Cornett (1982) and Cornett and Rigler (1984).

	1979				1980			
Lake	Z_H (m)	R_p ($mg \cdot m^{-2} \cdot yr^{-1}$)	NR ($mg \cdot m^{-3} \cdot d^{-1}$)	SPR ($mg \cdot m^{-3} \cdot d^{-1}$)	Z_H (m)	R_p ($mg \cdot m^{-2} \cdot yr^{-1}$)	NR ($mg \cdot m^{-3} \cdot d^{-1}$)	SPR ($mg \cdot m^{-3} \cdot d^{-1}$)
Central (M)	45.0	320	12.4±0.6	8.2±1.2	45.1	370	14.6±1.2	7.9±0.1
Massawippi	35.1	230	16.3±1.4	7.9±0.7	35.1	230	20.4±0.6	12.3±0.1
Orford	20.1	40	15.1±0.8	5.9±1.4	19.8	40	20.6±2.2	8.4±0.1
Lyster	—	—	—	—	16.4	40	21.2±0.8	5.7±0.3
Brompton	—	—	—	—	9.0	100	44.4±0.8	8.8±0.1
Stukley	—	—	—	—	8.9	30	35.4±0.8	6.4±0.1
North (M)	7.3	300	79.4±8.2	14.1±3.2	7.3	310	89.4±2.0	20.0±0.2
Little Brompton	—	—	—	—	6.3	60	67.6±4.6	10.6±0.2
Lovering	4.0	100	78.0±3.1	17.7±5.4	4.0	100	104.9±1.2	17.5±0.4
Magog	2.5	1070	216.1±7.6	57.3±5.6	3.7	1360	184.9±6.2	64.7±0.2
Fitch (M)	—	—	—	—	3.3	470	163.6±7.9	35.3±0.5
Truite	3.2	110	64.7±12.8	30.0±4.2	2.8	100	74.3±5.3	22.1±0.1

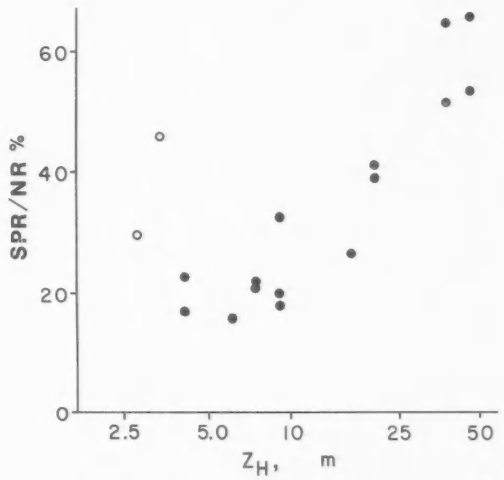

FIG. 4. Percentage of the total rate of oxygen respiration in the hypolimnion which occurred in the water column. Only lakes with mean summer phosphorus concentrations $<13\ \mu g \cdot L^{-1}$ are included in the comparison. The open circles are Lake Truite. Note log scale on *x*-axis.

that SPR controlled NR, while SOD varied very little. They postulated that SPR would increase as NR increased. The strong correlation between NR and SPR which we observed ($N = 19$, $R = 0.92$, $p < 0.001$) was consistent with this hypothesis. In lakes with similar hypolimnetic depth, but differing rates of sedimentation (measured by R_p), the fraction of the total hypolimnetic rate of respiration contributed by the water was also predicted to increase. Over the range of R_p values measured in this study, there was a significant nonparametric correlation between the ratio of SPR:NR and the estimated retention of phosphorus ($\tau = 0.7$, $p < 0.05$). The ratio of SPR:NR increased from 16 to 35%. However, this relationship explains <50% of the variance in the SPR:NR ratio. Cornett and Rigler (1979, 1980) and Charlton (1980) also postulated that in a group of lakes with similar surface water production and biomass, the proportion of NR attributed to SPR would increase in a semilogarithmic manner as the ratio of hypolimnetic water volume to sediment area increased. As predicted, the average fraction of the total respiration that occurred in the water column increased from ~20 to ~60% of NR as the mean depth of the hypolimnion increased from 4 to 45 m (Fig. 4). There was, however, one lake that did not fit the pattern of the other 11 lakes.

Much higher SPR values were measured in Lake Truite than in the other small oligotrophic lakes (Table 1; Fig. 4). The fraction of total respiration that occurred within the water column was also much larger than in other lakes. These observations may reflect lower rates of respiration in the sediments or algal photosynthesis in the hypolimnion. The second hypothesis is believed to be correct for two reasons. First, this was the only study lake in which >1% of the light at the lake's surface reached the maximum depth of the lake. Second, as summer progressed, oxygen concentrations in the upper strata of the hypolimnion increased. Because vertical eddy diffusivity measurements showed that there was no oxygen transported across the upper boundary of the hypolimnion of Lake Truite (R. J. Cornett and F. H. Rigler, unpubl. data), this increase must be due to photosynthesis.

Cornett and Rigler (1979, 1980) and Charlton (1980) also postulated that in a group of lakes with similar surface water productivity and similar hypolimnetic temperatures, the respiration rate of the sediments would not be significantly higher in shallow lakes than in deep lakes. Although this hypothesis cannot be tested rigorously until respiration by the sediments is measured directly, the difference between NR and SPR provides an estimate of the rate of oxygen consumption by the sediments. In the oligotrophic lakes that were studied, SOD was not correlated with the thickness of the hypolimnion ($N = 19$, $R = 0.15$, $p > 0.05$).

Although few other studies have measured SPR directly (Linsey and Lasenby 1985), many statements about the significance of respiration in the water column of lakes have been made (Table 2). The conflicting statements were usually based upon indirect estimates of SPR. Hutchinson (1957) placed dark bottles of hypolimnetic water in the hypolimnion of Lindsley Pond and Quassapang Lake for 1 wk. Since the change in oxygen concentration inside the bottles was approximately equal to the change within the hypolimnion, Hutchinson (1957)

TABLE 2. Published estimates of the areal hypolimnetic oxygen deficit (AHOD) and percent of the AHOD attributed to SPR (% SPR) by three methods of measurement: SPR was calculated as the difference between the AHOD and SOD measures (T-SOD); SPR was determined by the change in loss on ignition of particles collected in sediment traps (LOI); or SPR was calculated from the change in oxygen concentration in biological oxygen demand experiments (BOD).

Lake	AHOD ($mg \cdot m^{-2} \cdot d^{-1}$)	% SPR	Method	Source
Esrom	650	0	T-SOD	Hargrave 1971
Erie (central)	430	20	T-SOD	Burns 1976
Windermere	390	50	T-SOD	Lund et al. 1963
Dickey (south)	410	5	T-SOD	Lasenby 1975
Dickey (north)	190	56	T-SOD	Lasenby 1975
Dunham	180	60	T-SOD	Rich 1979
Grand	300	<0	T-SOD	Hayes and MacAuley 1959
Black Brook	210	<0	T-SOD	Hayes and MacAuley 1959
Copper	180	<0	T-SOD	Hayes and MacAuley 1959
Punchbowl	170	<0	T-SOD	Hayes and MacAuley 1959
Lauzon	—	0	LOI	Kleerekoper 1953
Mikolajki	—	4	LOI	Lawacz 1969
Jack	—	68	SPR	Linsey and Lasenby 1985
Linsley	—	100	BOD	Hutchinson 1957
Quassapaung	—	100	BOD	Hutchinson 1957

conclused that 100% of the oxygen consumption in the hypolimnion occurred in the water column. These SPR measures are much higher than the results obtained in any of the shallow lakes investigated during this study. Hayes and MacAulay (1959) reached the opposite conclusion to Hutchinson. They concluded that all hypolimnetic respiration occurred in the sediments after comparing rates of oxygen consumption in six small lakes with SOD values of mud cores measured in the laboratory. Kleerekoper (1953) and Lawacz (1969) also concluded that respiration in the hypolimnetic water column was not important. They observed that the loss of ignition (LOI) of seston collected in sediment traps, suspended at the upper boundary of the hypolimnion, was the same as the LOI of bottom sediments or trap material collected at the lake's maximum depth. From these observations they concluded that no respiration occurred in the water column. The results of Hutchinson (1957), Hayes and MacAulay (1959), Kleerekoper (1953), and Jawacz (1969) are difficult to interpret in light of our studies and those of others which show significant amounts of respiration occurring in the water and in the sediments.

Our results are consistent with those of Lund et al. (1963), Kimmel and Goldman (1976), Lasenby (1975), Rich (1979), and Linsey and Lasenby (1985). All of these authors measured the amount of oxygen consumed by the sediments and concluded that SOD was not sufficient to account for all of the oxygen depletion in the hypolimnion. However, Hargrave (1971) concluded that there was no respiration in the hypolimnetic water column of Lake Esrom. We cannot explain his observation. Based upon this study, 25–40% of the total amount of hypolimnetic oxygen consumed should be respired within the hypolimnetic water column of a shallow and highly productive lake, such as Lake Esrom.

Our measurements and those referenced above provide an empirical foundation for a conceptual model of sestonic and total hypolimnetic oxygen consumption. Variations in SPR within the hypolimnion and between lakes can be correlated with the in situ temperature and concentrations of organic matter. SPR is a significant fraction (15–66%) of the total hypolimnetic oxygen deficit. SPR increases in importance in deeper lakes. Although the variations in SPR can be positively correlated with lake depth, sediment respiration does not show a corresponding inverse relationship. Such a relation was expected if organic matter that was not decomposed in the water was decomposed in the sediments. Lake morphometry must alter the importance of sestonic respiration and it will change the fate of organic matter sedimented into the hypolimnion.

Acknowledgments

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Growth, Reproductive Output, and Energy Partitioning in Weathervane Scallops, *Patinopecten caurinus*, from British Columbia

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MacDonald, B. A., and N. F. Bourne. 1987. Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia. Can. J. Fish. Aquat. Sci. 44: 152–160.

Major differences in population growth and reproductive characteristics of *Patinopecten caurinus* were related to water temperatures and food sources associated with collection sites in the Strait of Georgia and off the west coast of Vancouver Island. Inshore populations were dominated by larger, faster growing scallops that apparently live longer than offshore scallops. Individuals from the inshore site exhibited enhanced reproductive output, total production, and turnover ratios compared with offshore scallops. Histological and stereological techniques were used to assess the annual reproductive cycle. Results revealed a single but protracted spawning period for inshore scallops beginning in mid-April and ending in mid-June whereas offshore scallops spawned during July and August. As scallops grew older there was a gradual shift in emphasis from growth to reproduction in addition to periods when gametogenesis proceeded at the expense of somatic tissue. The quantity of available energy invested in reproduction may restrict growth periods and limit the amount of growth for bivalve species from various habitats.

Les principales variations de la croissance démographique et des caractéristiques de reproduction de *Patinopecten caurinus* sont liées à la température de l'eau et aux sources alimentaires propres aux sites de collecte dans le détroit de Géorgie et au large de la côte ouest de l'île Vancouver. Les populations côtières sont surtout composées de gros pétoncles à croissance rapide qui vivent selon toute apparence plus longtemps que ceux qui vivent au large. Des individus recueillis dans la zone côtière ont montré une augmentation de la capacité de reproduction, de la production totale et des proportions de renouvellement par rapport aux pétoncles de la zone hauturière. Les auteurs ont utilisé des méthodes histologiques et stéréologiques pour déterminer le cycle de reproduction annuelle. Les résultats ont révélé une seule période de ponte prolongée en zone côtière, de la mi-avril à la mi-juin, tandis qu'en zone hauturière, les pétoncles se reproduisaient en juillet et août. Avec le temps, la reproduction a graduellement pris le pas sur la croissance en plus des périodes où la gamétogénèse se faisait aux dépens du tissu somatique. La quantité d'énergie disponible consacrée à la reproduction peut réduire les périodes de croissance et limiter la croissance des espèces de bivalve peuplant divers habitats.

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The weathervane scallop, *Patinopecten caurinus*, occurs only in the northeast Pacific from Alaska to California in water depths of 10–200 m (Bernard 1983a). Although weathervane scallops do not occur in large beds, sufficient quantities exist on scattered beds in southeast Alaska to support a small commercial fishery. In 1981 a bed of weathervane scallops was found off Oregon and 7620 t were landed that year, but they have declined to much lower levels since then (R. Starr, Oregon Fish and Wildlife, Newport, OR 97365, pers. comm.). Populations in British Columbia are small and scattered and there is no commercial fishery (Bourne 1969).

Sexes are separate in *P. caurinus* with no external signs of sexual dimorphism. Gonadal development in *P. caurinus* from Alaska occurs from October to May with a single spawning between June and mid-July (Hennick 1970). With the exception of some studies on reproduction (Hennick 1970), growth rates (Haynes and Hitz 1971), and physiology (Bernard 1983b), information on population characteristics and ecological production of *P. caurinus* is lacking.

Studies have been carried out on weathervane scallops in British Columbia to assess the feasability of establishing a commercial fishery and the potential for aquaculture (Bourne 1969). The objectives of this particular study were (1) to provide basic information on growth, population structure, and seasonal reproductive characteristics of unexploited populations of *P. caurinus* in British Columbia and (2) to determine whether conditions associated with offshore and inshore subtidal environments altered age-specific production of gametes and somatic tissue or patterns of partitioning available energy in this species.

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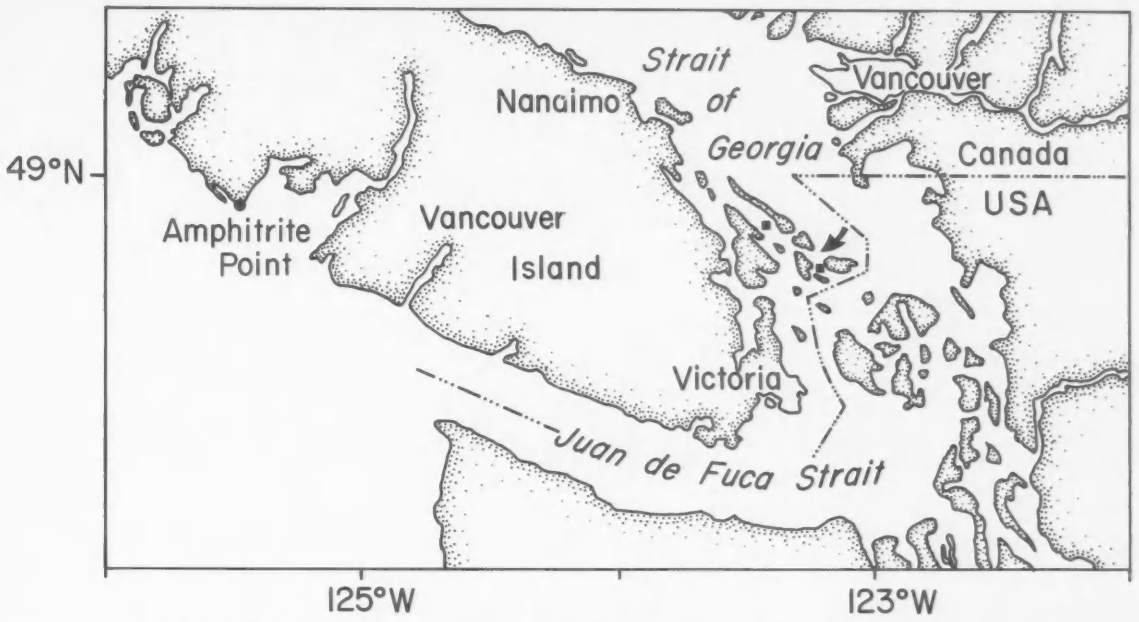

FIG. 1. Locations of collection sites in the Strait of Georgia (■) and 30 km off Amphitrite Point (●), Vancouver Island.

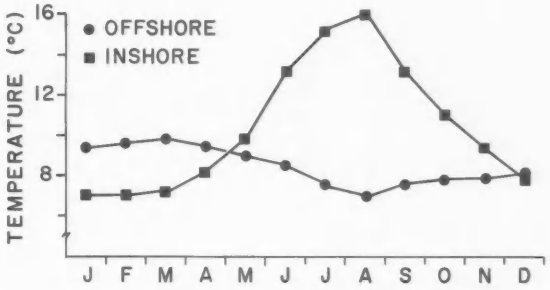

FIG. 2. Seasonal temperature cycles near the inshore sites in the Strait of Georgia (data from Hollister and Sandnes 1972) and the offshore site (data from Dodimead 1984).

Materials and Methods

Collection and Environmental Data

Samples of *P. caurinus* from inshore populations were collected from depths of 25–40 m in Plumper Sound and Tricomali Channel in the Strait of Georgia using Digby dredges (Fig. 1). Samples of scallops were collected from an offshore population at a depth of 110 m approximately 30 km off Amphitrite Point on the west coast of Vancouver Island using an otter trawl (Fig. 1). Inshore *P. caurinus* populations have been routinely sampled since 1968 on an approximately monthly schedule in some years and only between February and May, prior to spawning, in other years. Scallops from the offshore population were available in prespawning condition in June and postspawning condition in August during 1984 and 1985.

Water temperatures and salinities recorded daily at Porlier Pass in the Stait of Georgia and at Amphritrite Point on the west coast of Vancouver Island (Fig. 2) are considered representative of the collection sites. Surface water temperatures measured at Porlier Pass in the Strait of Georgia are considered indicative of conditions on the bottom at the scallop bed in Trincomali Channel, about 18 km from Porlier Pass, because there are strong currents and mixing in this area (Waldichuk 1957). Water temperatures off Amphitrite Point were taken at 100 m. Water temperatures near the inshore sites fluctuated seasonally with maxima in July and August and minimum values from December to March (Fig. 2). Temperatures at the offshore site showed little seasonal variation and were lower than those recorded for the inshore site except during the winter.

Growth

External growth rings on shells have been used to estimate ages of bivalves. These rings are formed annually and have been demonstrated to be useful age markers for several scallop species including *Placopecten magellanicus* (Stevenson and Dickie 1954), *Pecten maximus* (Mason 1957), *P. caurinus* (Haynes and Hitz 1971), *Patinopecten yessoensis* (Silina 1978), and *Swiftopecten swifti* (Ponurovski and Silina 1983). Prior to this study, the authenticity of annual growth rings for *P. cvaurinus* had not been establshed. Shells were sent to A. V. Silina who verified the annual nature of the rings by examining the microstructure of the external shell surface and oxygen isotope analysis of the valves.

Gonad and somatic tissue were removed and weighed separately to the nearest 0.01 g. Measurements of shell height (maximum distance between the hinge and ventral margin) were recorded to the nearest 1.0 mm using venier calipers. Mean shell heights for each age class were estimated using the von Bertalanffy equation

$$H_t = H_\infty [1 - e^{-k(t-t_0)}]$$

where H_t is shell height at time t, H_∞ is the mean asymptotic height, k is the Brody growth coefficient, and t_0 is a parameter representing time when height equals zero.

Polynomial regression may have an advantage over the forced asymptotic behaviour of the von Bertalanffy equation when describing somatic tissue growth (MacDonald and Thompson 1985a) and was therefore used in this study to describe the relationship between somatic weight and age to provide a mean weight for each age class. Polynomial regression may be described by the following equation:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 \ldots \beta_m x^m + \epsilon$$

where $\beta_0, \beta_1, \ldots, \beta_m$ are population parameters, y is the somatic weight for a given age, and ϵ is random error at observation x (N.B. $\beta_0 = \alpha$ in Zar's (1984) notation). According to Neter et al. (1983), multicollinearity in this type of regression can be reduced by replacing values of x (age) by $(x - \bar{x})$.

Values for gonad, somatic, and shell weight relationships with shell heights were fitted to the allometric equation $y = ax^b$ where y = predicted weight at shell height x and a and b = fitted parameters used to obtain mean weights and calculate production estimates for each age class. A linear form of the allometric equation $y = ax^b$ may be obtained by transforming both variates to logarithms and fitting the data by least squares regression to a straight line $\log_{10} y = \log_{10} a + b \log_{10} x$ (Snedecor and Cochran 1972). The General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc.) was used to calculate and compare regressions and for analysis of variance, and the Nonlinear procedure (NLIN) was used to construct the von Bertalanffy models.

Production

Total production consists of several components:

$$P = \mathrm{Pg} + \mathrm{Pr} + \mathrm{Ps} + P_{\mathrm{by}}$$

where Pg = energy invested in somatic tissue growth, Pr = energy released as gametes, Ps = energy incorporated in the organic matrix of the shell, and P_{by} = energy spent on byssus formation. Byssus production by *P. caurinus* was not considered important in this study. Numerous field and laboratory observations of *P. caurinus* have shown that larger animals, 50 mm shell height and larger, rarely attach by byssus (N. Bourne, unpubl. obs.).

Estimates of annual somatic tissue production (Pg) and shell production (Ps) were obtained by calculating increments in somatic tissue and shell weight W between consecutive year classes ($W_{(x+1)} - W_x$). Gamete production (Pr) for each age class was estimated from weight loss of the gonad on spawning calculated from the prespawning gonad weight versus shell height regressions.

To facilitate comparisons between published values and those recorded for *P. caurinus*, production estimates from this study were converted to energy equivalents using conversion factors derived largely from work on the giant scallop *P. magellanicus*. For example, 1 g dry weight gametes = 26.0 kJ (MacDonald and Thompson 1985b), 1 g dry weight somatic tissue = 24.5 kJ (Thompson 1977), 1 g organic matter in the shell (*Scrobicularia plana*) = 21.1 kJ (Hughes 1970), and dry weight/wet weight conversion for scallop gonads and somatic tissue = 0.20 (unpubl. data). Annual increments in shell weight for each age class were multiplied by the organic content of the shell (1.3%) (unpubl. data) to estimate the amount of organic material in these increments before converting to energy equivalents.

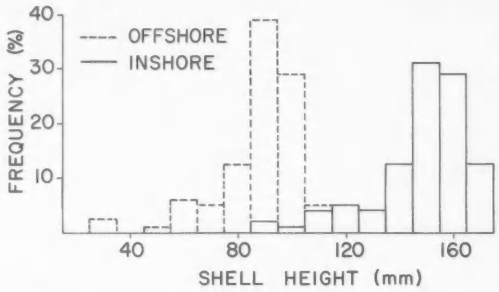

FIG. 3. Size frequency distributions for inshore and offshore populations of *Patinopecten caurinus*.

Reproductive Cycle

Pieces of gonad approximately 1 × 1 × 0.5 cm were excised from 24 scallops (12 female, 12 male) in each monthly sample from the inshore population. Gonad tissues were preserved in Davidson's fixative for a minimum of 48 h before processing through an ascending alcohol series and xylene prior to embedding in Paraplast. Sections (5–6 μm) were cut on a microtome and stained with hematoxylin and eosin.

The proportions of the gonad which are occupied by gametes, connective tissue, lumen space in the follicle, or other cell types are referred to as volume fractions. Volume fractions were estimated using point count and stereological techniques originally described by Weibel and Elias (1967), Freere (1967), and Briarty (1975) and later modified for use with mussel and scallop gonadal tissue (Lowe et al. 1982; MacDonald and Thompson 1986a). Mean monthly values of volume fractions for each of six males and six females were obtained by examining five random fields for each histological section using a Zeiss compound microscope fitted with a Weibel II eyepiece graticule (Graticules Ltd.) at 160× magnification. Preliminary observations revealed that the gonad of *P. caurinus* is structurally homogeneous like that of *Mytilus edulis* (Lowe et al. 1982) and *P. magellanicus* (MacDonald and Thompson 1986a).

Gonad indices, calculated by dividing gonad weights by the weight of remaining soft tissues, have been commonly used to describe quantitatively reproductive cycles (Giese and Pearse 1974).

Results

Size and Age Distribution

Inshore scallop populations were dominated by large individuals with shell heights between 150 and 160 mm representing scallops over the age of 9 yr (Fig. 3, 4). Less than 20% of the individuals collected were smaller than 130 mm (5 yr old), while there were no individuals less than 80 mm in height (3 yr of age). In contrast, the offshore population consisted mainly of scallops between 90 and 100 mm in shell height ranging in age from 4 to 6 yr with no individuals larger than 115 mm or 8 yr of age. Young individuals (<3 yr) were not abundant at the offshore site either but several small size classes were represented in the samples.

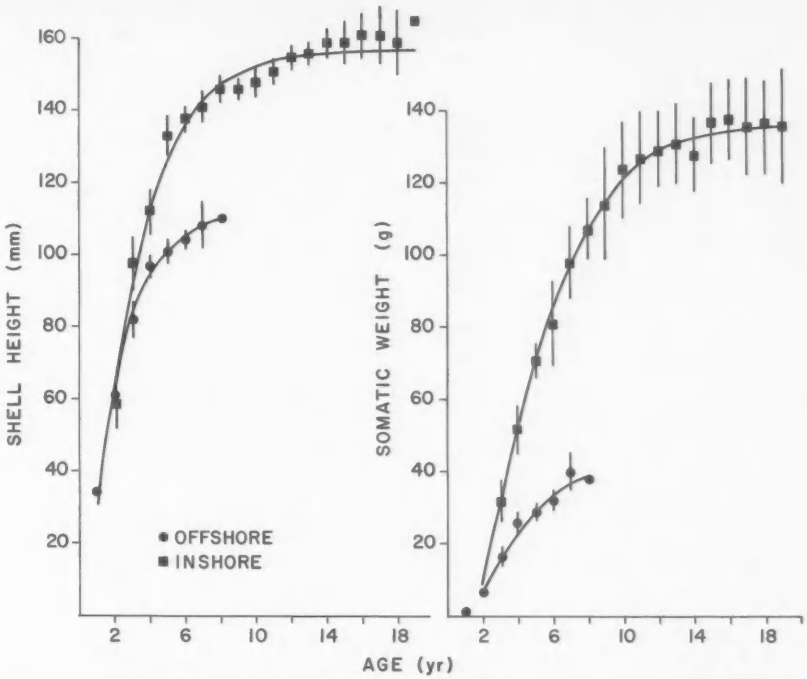

FIG. 4. Age-specific shell heights fitted to von Bertalanffy equations and somatic weights fitted to polynomials for inshore and offshore populations of *Patinopecten caurinus*. Plotted estimates represent mean height or weight and 95% confidence intervals with the $\bar{x}$ values used for polynomial equations equal to 4.5 (offshore) and 12.3 (inshore).

Growth Rates

With the possible exception of the first year or two of growth, when little information was available, mean shell height for each age class was consistently greater for inshore scallops (Fig. 4). Asymptotic shell heights (H_∞) were also much lower for offshore scallops, but the relative rate (k) at which the latter reached their respective asymptotic size was greater for this population (Table 1).

Due to the probability of seasonal differences in somatic weight, only scallops collected in June from inshore sites were used for comparative purposes corresponding to the months when offshore scallops were available. Mean somatic weight for each age class was also much greater for inshore scallops (Fig. 4). As shown by an analysis of covariance, individuals from the inshore population had heavier shells (slopes, $t = 11.2$, $P < 0.001$) and greater somatic weights (slopes, $t = 2.91$, $P < 0.01$) than scallops with the same shell height from the offshore population regardless of age or growth rate.

Reproduction

Seasonal changes in gametogenic activity could only be assessed for the inshore population of *P. caurinus*. Gametes seen in histological sections of scallop gonads have been classified either as developing (early stages) or mature (ripe gametes), although separation into the two categories is more difficult for females (MacDonald and Thompson 1986a).

Gamete volume fraction (GVF) values for total gametes (TG) increased steadily during the autumn and winter months (Fig. 5), reaching a maximum of approximately 90% in April before the single major spawning which occurred in late April or early May. Gonads without any gametes were not observed until July and August, indicating a complete but protracted spawning period of about 2 mo for this species. Mature gamete (MG) estimates for males had a pattern similar to TG values, reaching a maximum close to 75% before dropping to zero during July and August. However, MG for females remained near zero until January and rapidly increased to a maximum of approximately 85% prior to spawning in April. Total gamete values calculated separately for males and females (not shown) were much higher for males between November and January due to apparently faster gamete maturation rates in males. Total gamete values are the only descriptions of gametes that are directly comparable between males and females because the subjectivity associated with assigning them to categories is eliminated.

The proportion of developing gametes increased to a maximum of 40% in January before declining, owing to a rate of maturation for present gametes that exceeded development rates of new gametes. As percentages of gametes increased the follicles became densely packed resulting in a decline and eventual disappearance of lumen space immediately prior to spawning. Males and females displayed similar patterns for connective tissue values, reaching a peak of 40% after spawning and declined to minimum values of 10% before spawning.

Seasonal gonad indices have been described for *P. yessoensis* (Ventilla 1982), *Chlamys islandica* (Skreslet and Brun 1969), and *Chlamys varia* (Shafee and Lucas 1980). Values for both GVF and gonadosomatic indices (GSI) for inshore *P. caurinus* reached maximums in early May before

TABLE 1. Summary of parameters for shell height data (mm) presented in Fig. 4 fitted to the von Bertalanffy equation $H_t = H_\infty [1 - e^{-k(t - t_0)}]$ where H_t = height at age t, H_∞ = mean asymptotic height, k = Brody growth coefficient, and t_0 = a parameter representing time when H_t = zero. Parameters for somatic weight data (g) presented in Fig. 4 fitted to polynomial regression equations $y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$ where x = age of scallop (yr), y = predicted somatic weight, and $\beta_0, \ldots, \beta_3$ = population parameters. Also presented are parameters describing the relationships between weights (soma, gonad, and shell) and shell heights using the allometric equation $\log y = \log a + b \log x$, where x = shell height and y = predicted weight for a scallop of given size. All regressions are highly significant ($P \ll 0.001$).

	Inshore					Offshore				
	H_∞	k	t_0	n	r^2	H_∞	k	t_0	n	r^2
Shell height vs. age (von Bertalanffy)	156.8	0.39	0.72	176	0.96	112.4	0.50	0.35	80	0.92
	β_0	β_1	β_2	β_3	n	r^2	β_0	β_1	β_2	β_3	n	r^2
Somatic weight vs. age (polynomial)	130.1	2.43	−0.50	0.04	176	0.63	26.00	5.80	0.59	—	80	0.85
	log a		b		n	r^2	log a		b		n	r^2
Somatic weight vs. height	−3.48		2.52		50	0.83	−4.42		2.94		80	0.98
Gonad weight vs. height (prespawning condition)	−4.91		2.91		100	0.66	−6.85		3.65		47	0.84
Shell weight vs. height (postspawning condition)	−6.81		3.67		25	0.68	−6.00		3.02		33	0.89
Shell weight vs. height	−2.02		1.91		212	0.89	−3.83		2.72		80	0.97

declining rapidly due to spawning (Fig. 6).

Gonad weight for a "standard" inshore scallop of 140 mm shell height increased to a maximum in late April before rapidly declining due to spawning and then slowly decreased over the summer months (Fig. 7; Table 2). Somatic weight (140 mm scallop) declined during the latter stages of the gametogenic cycle (April−May) until spawning was complete and then rapidly increased to a maximum in July and remained high during the summer, while reproductive activity was low.

Production

Gamete production (Pr) for both populations increased steadily with age until an asymptote was reached (Fig. 8). Somatic production (Pg) in the offshore population increased during the earliest years, reached a maximum at age 3,and declined steadily until age 8. Values of Pg for inshore scallops declined rapidly between ages 3 and 14 and then decreased slowly until age 19. Somatic production and Pr were greater for inshore scallops than for scallops of equivalent age from the offshore population. Inshore scallops had heavier gonads than offshore scallops of the same size despite being younger (ANCOVA, slopes, $t = 2.33$, $P < 0.02$).

Reproductive output (Pr), biomass (B), and reproductive effort (RE) were all increasing functions of age during the lifespan, while turnover ratio (P/B) was a decreasing function of age (Table 3). Maximum values for somatic (Pg) and shell production (Ps) were reflected in corresponding peaks in total production (P) which generally declined with age because Pg and Ps declined faster than Pr increased. Every measured type of production or index presented in Table 3 was greater for inshore scallops than for those of the same age collected offshore except for RE at age 8. Inshore scallops were more productive per unit weight despite their larger body size.

Discussion

Slower shell growth rates have been observed for offshore populations of *P. caurinus* when compared with scallops collected from the inshore waters of the Strait of Georgia (this study; Haynes and Hitz 1971). In contrast with the well-mixed, warmer water in some parts of the Strait of Georgia, rich in phytoplankton during summer (Waldichuk 1957; Harrison et al. 1983), the deeper offshore site is characterized by a relatively cool, stable water mass, which may be below the productive euphotic zone (Lane 1963). Elevated water temperatures at the inshore site during summer months, when phytoplankton was relatively abundant, may be equally responsible for faster growth rates as any differences in food quantity or quality that may exist between offshore and inshore sites.

Reduced growth rates for scallops from deeper water have been reported for *P. magellanicus* (Brannen 1940; Caddy et al. 1970; Posgay 1979; MacDonald and Thompson 1985a), *Hinnites multirugosus* (Leighton 1979), and *P. maximus* (Mason 1957). Alternatively, there have been examples of negligible differences in shell growth related to water depth for *Chlamys opercularis* (Richardson et al. 1982), *P. maximus* (Gruffydd 1974), and *P. magellanicus* (MacDonald and Thompson 1985a). All of these authors attributed growth variations, or lack of them, to differences or similarities in food and temperature conditions. Poorer environmental conditions at offshore sites are also probably responsible for the reduction in maximum size achieved by *P. caurinus* in that habitat.

Size frequency distributions that contain relatively few small scallops have often been described for *P. magellanicus* (Caddy et al. 1970; Jamieson et al. 1981; D'Amours and Pilote 1982; MacDonald and Thompson 1986b) and may be a common characteristic among populations of large pectinid species, including *P. caurinus*. There was almost no overlap in size classes between inshore and offshore samples of *P. caurinus*, with inshore scallops apparently living longer (Haynes and Hitz 1971; this study). Differences in longevity may be related to degree of infestation by organisms that bore into the shell such as sponges or annelids, commonly observed in shells of larger

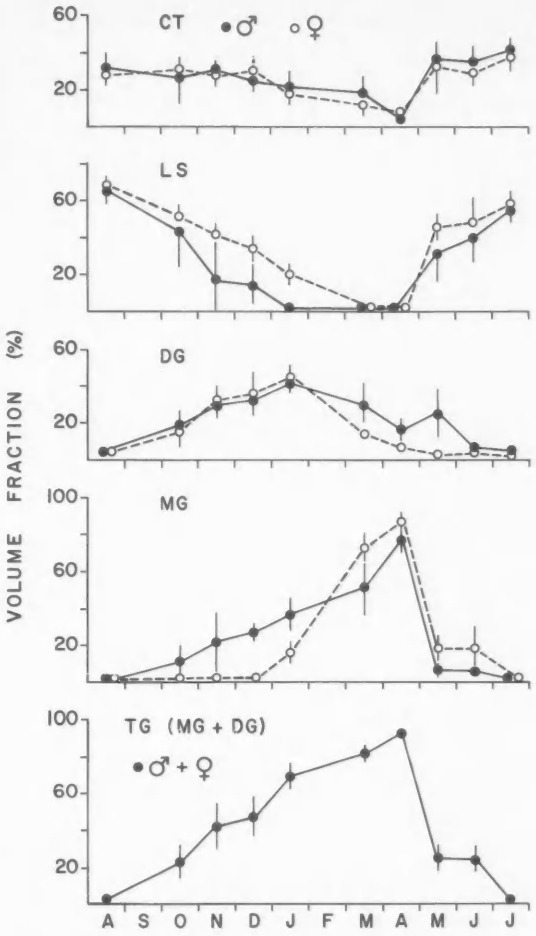

FIG. 5. Gamete volume fraction for connective tissue (CT), empty follicle or lumen space (LS), developing gametes (DG), plus mature gametes (MG) plotted separately for inshore male and female *Patinopecten caurinus*. Total gametes (TG = MG + DG) for male and female samples combined are also included. Values represent means and 95% confidence intervals.

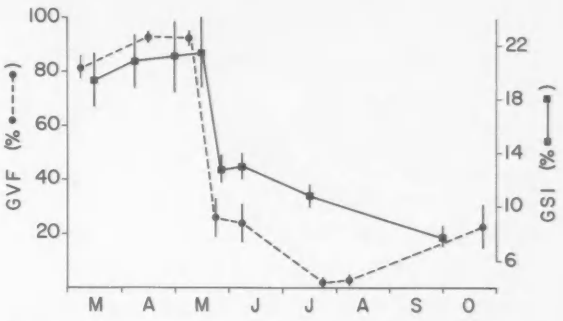

FIG. 6. Determination of reproductive activity and spawning period for inshore *Patinopecten caurinus* by monitoring seasonal changes in gamete volume fraction (GVF) for total gametes (males and females combined) and gonadosomatic index (GSI) calculated as gonad weight/somatic weight × 100. Values represent means and 95% confidence intervals.

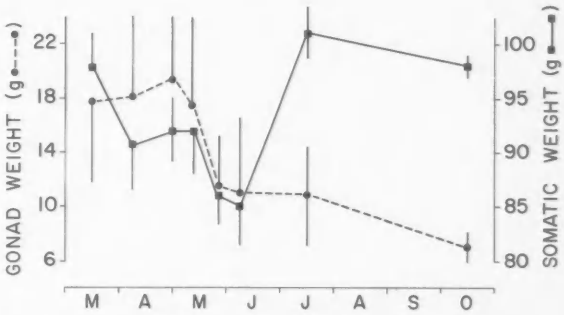

FIG. 7. Seasonal changes in mean somatic tissue and gonad weights (±95% confidence intervals) for a standard inshore *Patinopecten caurinus* 140 mm in shell height.

scallops from offshore areas but not in scallops from the Strait of Georgia (Haynes and Hitz 1971; this study). Boring organisms may weaken the host or increase the probability of mortality by direct or indirect means as reported for *P. magellanicus* (Medcof and Bourne 1964) and *Aequipecten gibbus* (Wells and Wells 1962).

Greater somatic tissue weights were also observed for scallops from the inshore site compared with offshore scallops of the same age. Reduced body weights for scallops from less favourable deeper water environments have been reported for *H. multirugosus* (Leighton 1979) and *P. magellanicus* (MacDonald and Thompson 1985a). Gonad weight in bivalves is well correlated with body size, so age-specific reproductive output was much greater for inshore scallops. The combination of greater gamete and soma production for inshore *P. caurinus* resulted in age-specific estimates of total production that exceeded those obtained for offshore scallops by a factor of 3 or 4. Enhanced reproductive output and total production under better environmental conditions have been described for *M. edulis* (Bayne and Worrall 1980) and *P. magellanicus* (MacDonald and Thompson 1985b).

Mean gonadosomatic indices, calculated prior to spawning in April for inshore and June for offshore *P. caurinus* populations, were 21.4% (±2.6 95% C.I.) and 13.1% (±2.2 95% C.I.), respectively. Skreslet and Brun (1969) also reported lower GSI values for *C. islandica* from deeper water than in scallops collected from shallow water, which they attributed to differences in food sources. Gonadosomatic indices are easier to estimate than measurements of GVF for describing reproductive activity and can be reasonably accurate in determining major spawning periods. However, if more detailed information is required, GSI estimates cannot differentiate between spent gonads that contain ripe residual gametes and gonads with small developing gametes, nor does the GSI reveal whether the gonad contains any gametes at all. Misinterpretation concerning reproductive activity may result, e.g. higher values for GSI were observed in July, when there were essentially no gametes in the gonad (determined histologically by GVF), than in October when gametogenic activity had increased again (Fig. 6).

As bivalves grow older there is a shift in allocation of production from an early emphasis on soma and shell to an even-

TABLE 2. Allometric relationship $\log y = \log a + b \log x$, where x = shell height (mm) and y = predicted weight (g), describing seasonal changes in the relationship between somatic weight or gonad weight and shell height of inshore scallops. All regressions are highly significant ($P \ll 0.001$).

Sample date (1984)	Somatic weight				Gonad weight			
	log a	b	F	r^2	log a	b	F	r^2
March 16	−4.19	2.88	350.3	0.88	−3.82	2.11	17.6	0.27
April 5	−3.45	2.52	1048.6	0.96	−3.87	2.39	59.7	0.55
April 30	−4.26	2.90	349.4	0.89	−3.11	2.05	19.4	0.32
May 10	−3.36	2.48	466.9	0.91	−4.08	2.48	30.3	0.39
May 28	−3.41	2.49	131.0	0.78	−3.34	2.05	18.4	0.33
June 6	−3.48	2.52	232.3	0.83	−4.49	2.58	53.9	0.53
July 11	−3.96	2.78	212.5	0.82	−5.04	2.83	45.5	0.49
October 2	−3.01	2.33	75.4	0.71	−4.28	2.39	31.3	0.50

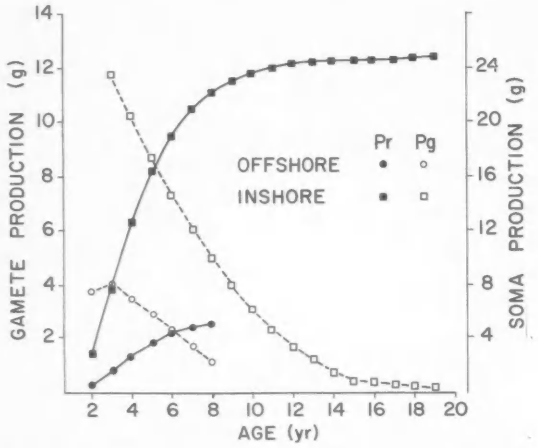

FIG. 8. Annual gamete (Pr) and somatic production (Pg) for each age class of scallops from offshore and inshore populations of *Patinopecten caurinus*. Males and females were combined for estimates because there was no significant difference (ANCOVA) in somatic weight (slope, $t = 0.54$, $P < 0.59$; elevation, $t = 0.56$, $P < 0.58$) or prespawning gonad weight (slope, $t = 0.10$, $P < 0.92$; elevation, $t = 0.17$, $P < 0.87$) relationships with shell height.

TABLE 3. Age-specific estimates of shell height (mm) and energy equivalents for mean somatic weight of biomass (B; kJ) and total production (P; kJ·yr^{-1}) partitioned into somatic production (Pg; kJ·yr^{-1}), shell organics (Ps; kJ·yr^{-1}), and reproductive output (Pr; kJ·yr^{-1}). Mean turnover ratios (P/B) and reproductive effort (RE) ([Pr/P] × 100) for inshore and offshore scallops are also included.

Age	Shell height	B	Pg	Pr	Ps	P	P/B	RE
				Offshore				
2	63.1	42.5	36.9	1.3	2.7	40.9	0.96	3.2
3	82.5	81.9	39.4	4.1	3.4	46.9	0.57	8.7
4	94.3	115.6	33.7	7.0	3.0	43.7	0.38	16.0
5	101.4	143.4	27.8	9.5	2.1	39.4	0.27	24.1
6	105.7	165.5	22.1	11.1	1.5	34.7	0.21	32.0
7	108.4	181.7	16.3	12.4	0.8	29.5	0.16	42.0
8	109.9	192.2	10.5	13.2	0.6	24.2	0.13	54.1
				Inshore				
2	—	—	—	—	—	—	—	—
3	92.4	158.7	116.1	20.5	8.0	144.6	0.91	14.2
4	113.2	158.9	100.2	32.8	7.0	140.0	0.54	23.4
5	127.3	344.6	85.7	42.6	4.2	132.5	0.38	32.2
6	136.8	416.8	72.2	49.6	3.8	125.6	0.30	39.5
7	143.3	476.7	59.9	54.4	3.4	117.7	0.25	46.2
8	147.6	525.6	48.8	57.8	2.7	109.3	0.21	52.9
9	150.6	564.5	38.9	59.9	2.3	101.1	0.18	59.3
10	152.6	594.7	30.2	61.5	1.9	93.6	0.16	65.7
11	154.0	617.3	22.6	62.6	1.5	86.7	0.14	72.2
12	154.9	633.5	16.2	63.2	1.1	80.5	0.13	78.5
13	155.5	644.5	11.0	63.6	0.6	75.2	0.12	84.6
14	155.9	651.5	7.0	63.9	0.4	71.3	0.11	89.6
15	156.2	655.6	4.1	64.2	0.4	68.7	0.10	93.5
16	156.4	658.0	2.4	64.3	0.3	67.0	0.10	96.0
17	156.5	659.9	1.9	64.4	0.2	66.5	0.10	96.8
18	156.6	661.5	1.6	64.5	0.2	66.3	0.10	97.3
19	156.7	662.5	1.0	64.6	0.1	65.7	0.10	98.3

tual commitment of almost all available energy to gamete production. The corresponding increase in reproductive effort with advancing age for *P. caurinus* has also been reported for several other bivalves, but the individual response to variable environmental factors has received less attention (Bayne and Newell 1983). Offshore scallops responded to apparently less favourable environmental conditions by altering their energy partitioning pattern to reduce amounts of energy spent on reproduction in comparison with investments in somatic tissue. Reduced reproductive effort under poor or stressful conditions has been observed for *M. edulis* (Bayne et al. 1983) and *P. magellanicus* (MacDonald et al. 1987) and is consistent with predictions by Goodman (1979).

Maximum recorded values of reproductive effort for offshore scallops were approximately 50%, indicating that these scallops were still putting half their production into growth. Most long-lived bivalve species, including inshore *P. caurinus*, are partitioning far less than 10% of their production into growth by the time they reach their maximum age (Bayne and Newell 1983). This is certainly true of *P. magellanicus* (MacDonald et al. 1987) from poor environmental conditions not unlike those experienced by offshore *P. caurinus*. This relatively high commitment to growth for apparently senescent animals may be further evidence that the natural life expectancy (<8 yr) for offshore scallops in this study has been prematurely truncated by undetermined factors that may include boring organisms.

The trend of decreasing turnover ratios with advancing age seen for *P. caurinus* in this study has also been described for *C. islandica* (Vahl 1981), *C. varia* (Shafee and Conan 1984),

and *P. magellanicus* (MacDonald and Thompson 1985b). This decline occurs because older bivalves devote a greater proportion of their energy acquisition to support metabolism than do younger individuals, resulting in less energy available for production relative to body size (Valh 1981; Bayne 1984). Turnover ratios may essentially become independent of age for long-lived species such as *P. caurinus* (inshore) if production, mainly due to increasing reproductive output, increases with age at the same rate as body weight or biomass, as reported for *M. edulis* (Thompson 1984a) and *P. magellanicus* (MacDonald and Thompson 1985b). Despite their larger biomass, for any given age the turnover ratio is greater for inshore than offshore weathervane scallops due to their substantially greater gonad output.

The major spawning period for *P. caurinus* from the Strait of Georgia is late April and May, earlier than recorded for the same species from Alaska (Hennick 1970). Although not determined in this study, stored energy reserves may provide the necessary energy to fuel rapid gamete development between January and April rather than from recently assimilated external food sources which are at reduced levels before the spring bloom in May or June (Harrison et al. 1983). Rapid maturation of gametes occurred immediately after the spring bloom in *M. edulis* from the Baltic (Kautsky 1982) and Newfoundland (Thompson 1984b), in *C. islandica* from Norway (Sundet and Lee 1984), and in *P. magellanicus* from Newfoundland (MacDonald and Thompson 1986a). The spring bloom may represent a significant contribution to the organism's annual energy budget, and various suspension-feeding bivalve species may adopt different strategies to utilize this energy most efficiently. Some species experience active gametogenesis simultaneously with accumulation of energy reserves before they spawn during summer after the spring bloom, referred to as opportunistic species (Bayne 1976). Utilization of accumulated energy at this time may enable adults to complete gametogenic phases, increase rates of gamete maturation, and/or increase quantities of gametes produced. Alternatively the timing of spawning for conservative species may coincide with the spring bloom to improve the chances that planktotrophic larvae will encounter proper food conditions necessary for development, growth, and survival over warm summer months (Bayne 1976).

Somatic weight declines as gametogenic activity intensifies and subsequently increases again during relatively inactive gametogenic phases, which emphasizes the large energy investments in reproduction by the weathervane scallop. In terms of available energy, completion of the reproductive cycle apparently takes priority over growth at the expense of body tissue. This has also been observed in *C. varia* (Shafee 1980), *Macoma balthica* (DeWilde 1975; Nichols and Thompson 1982), and *P. magellanicus* (MacDonald and Thompson 1986a). The inverse relationship between body weight and gametogenic activity may vary between species of bivalves and be influenced by local food and temperature conditions. For example, *M. balthica* may continue to grow as gamete development proceeds if lower temperatures reduce the energy required for metabolic processes (DeWilde 1975). The annual period of growth for bivalves may be restricted by timing of the reproductive cycle and by energy demands of reproduction.

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Changes in the Immune System of Coho Salmon (*Oncorhynchus kisutch*) during the Parr-to-Smolt Transformation and after Implantation of Cortisol[1]

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Maule, A. G., C. B. Schreck, and S. L. Kaattari. 1987. Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-to-smolt transformation and after implantation of cortisol. Can. J. Fish. Aquat. Sci. 44: 161–166.

The primary immune response of coho salmon (*Oncorhynchus kisutch*), as assessed by the production of splenic antibody-secreting cells (plaque-forming cells, PFC) after an injection of *Vibrio anguillarum* O-antigen, decreased during smoltification. This period was marked by increases in gill Na^+-K^+-adenosine triphosphatase (ATPase) activity and plasma thyroxine and cortisol titers. Numbers of leucocytes relative to erythrocytes in peripheral blood and splenic lymphocytes relative to fish body weight were also reduced. Fish reared at normal hatchery density (approximately 2 fish·L^{-1}) appeared to have reduced rates of development and higher numbers of PFC than fish reared at one-third normal density. Moreover, in fish changed from normal density to low density 2 wk before sampling, ATPase activity and plasma thyroxine levels were equal to those in fish reared continuously at normal density, but plasma cortisol levels and PFC were equal to those in fish reared at low density. Fish with cortisol implants had higher plasma cortisol titers, reduced numbers of splenic PFC, splenic lymphocytes, and circulating leucocytes, and greater mortality when fish were exposed to *V. anguillarum*.

La réaction immunologique primaire du saumon coho (*Oncorhynchus kisutch*), déterminée par la production de cellules spléniques sécrétées d'anticorps (cellules génératrices de plaque (CGP)) après l'injection d'antigène 0 de *Vibrio anguillarum*, a diminué pendant la smoltification. Cette période a été marquée par des augmentations de l'activité du Na^+-K^+-adénosine triphosphatase (ATPase) dans les ouïes et des titres de thyroxine et de cortisol dans le plasma. De plus, le nombre de leucocytes par rapport au nombre d'érythrocytes dans le sang périphérique et la quantité de lymphocytes spléniques par rapport au poids corporel ont montré une baisse. Chez les poissons élevés à une densité normale en écloserie (environ 2 poissons·L^{-1}), les taux de développement étaient plus faibles et le nombre de CGP plus élevé que chez les poissons gardés à un tiers de la densité normale. De plus, chez les poissons transférés d'une densité normale à une faible densité 2 sem avant l'échantillonnage, l'activité de l'ATPase et les teneurs en thyroxine du plasma étaient semblables à ceux des poissons gardés sans interruption à une densité normale; par contre, les teneurs en cortisol du plasma et le nombre de CGP étaient les mêmes que ceux de poissons élevés à faible densité. Chez les poissons qui ont fait l'objet d'implantations de cortisol, les titres de cortisol plasmatique étaient plus élevés, le nombre de CGP spléniques, de lymphocytes spléniques et de leucocytes circulants était plus faible et la mortalité des poissons exposés à *V. anguillarum* était plus grande.

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Smoltification of juvenile anadromous salmonids prepares them for successful entry into salt water, and is marked by changes in behavior, morphology, and physiology (Hoar 1976; Folmar and Dickhoff 1981). Included in these are changes in plasma levels of cortisol (Specker and Schreck 1982; Barton et al. 1985), which has been shown to affect the mammalian immune system (Ader 1981; Besedovsky et al. 1985; Blalock et al. 1985). The administration of corticosteriods to fish can alter the morphology of immune organs (Chilmonczyk 1982; Peters and Schwarzer 1985), suppress immune function (Anderson et al. 1982), and reduce resistance to disease (Pickering and Duston 1983). Moreover, alteration in feeding behavior of salmon during smoltification and after release from a hatchery (Paszkowski and Olla 1985) may alter the nutritional value of their diet, which may in turn alter immune competence (Blazer and Wolke 1984a, 1984b). Developmental and environmental changes associated with smoltification may thus result in alterations in immune function of salmonids when they begin migrating to the ocean.

In the experiments discussed here, we assessed the immune system of juvenile coho salmon (*Oncorhynchus kisutch*) before and during smoltification to determine if there was a period of immunosuppression accompanying other physiological

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changes during smoltification. Futhermore, we examined the possible role of cortisol as an immunosuppressor by evaluating the effects of treatment with cortisol.

General Methods

Four stocks of juvenile coho salmon were used during these experiments which were conducted during November – July of the year in which the fish underwent smoltification. All fish were held at either the Oregon State University Fish Disease Laboratory or Smith Farm Experimental Hatchery, Corvallis, Oregon. Both facilities are suppied with fish-pathogen-free well water that is 12.0 ± 0.5°C throughout the year. Fish were fed Oregon Moist Pellet at 1–2% body weight daily, except on the day when experiments occurred.

The passive hemolytic plaque assay to *Vibrio anguillarum* O-antigen (VOA), as described by Kaattari and Irwin (1985), was used to assay the immune system. Briefly, we extracted O-antigen from *V. anguillarum* (LS-174) using the ethanol-incubation, acetone-drying method described by Anderson et al. (1979). Fish were anesthetized in tricaine methanesulfonate (MS-222) at a concentration of 50 $mg \cdot L^{-1}$ and were injected intraperitoneally with 0.1 mL of a 100 $\mu g \cdot mL^{-1}$ suspension of powdered antigen in phosphate-buffered saline (PBS). Seventeen days after immunization, the fish were killed by a blow to the head and lymphoid organs were removed by dissection. Single-cell suspensions of individual organs were washed twice by centrifugation and resuspended in L-15 medium. Lymphocyte viablity, determined by trypan blue exclusion and hemacytometer cell counts, was always at least 90% and usually exceeded 95%. Cells secreting anti-VOA antibody (i.e. plaque-forming cells, PFC) were enumerated using a modification of the Cunningham plaque assay (Cunningham and Szenberg 1968). A mixture containing 100 µL of cell suspension, 25 µL of diluted steelhead (*Salmo gairdneri*) serum, and 25 µL of a 10% dilution of sheep red blood cells (SRBC) that had been coated with VOA was deposited into a slide chamber. The chambers were then sealed with wax and incubated for 1–3 h at 15°C. We counted PFC using 7–30 magnification. At least two non-immunized fish were also assayed for PFC during each assay to determine nonspecific SRBC lysis. These controls ranged from 0 to 10 PFC per million lymphocytes, but never exceeded 4% of PFC in immunized fish.

Smears of peripheral whole blood were air-dried, fixed in ethanol, and stained with Geimsa stain. White blood cells (WBC) — lymphocytes, polymorphonucleocytes, and thrombocytes — were enumerated on the basis of descriptions by Anderson (1974) and Yasutake and Wales (1983).

Plasma cortisol and thyroxine (T_4) titers and gill Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase) activity have been shown to increase at the time when smoltification is believed to be happening (Zaugg et al. 1972; Dickhoff et al. 1978; Specker and Schreck 1982). Although the functional significance of these changes has not been determined, we monitored these three varibles in order to have a general idea of when smoltification occurred. On the same day that fish were immunized, other fish (n = 16–20) were rapidly netted from their home tank and immobilized in 5 L of water containing 1 g of MS-222, a dose that has been shown to inhibit stress-related increase in plasma cortisol titers (Strange and Schreck 1978). After severing the caudal peduncle, we collected blood from the dorsal aorta into heparinized capillary tubes. After centrifugation, plasma was collected, stored at −20°C, and later assayed for cortisol and T_4 using radioimmunoassays described by Redding et al. (1984) and Dickhoff et al. (1978), respectively. Gill filaments were dissected from these fish and stored in sucrose buffer at −70°C. The Na^+-K^+-ATPase activity was determined for us by Dr. W. Zaugg (National Marine Fisheries Service, Cook, WA) by the method of Zaugg et al. (1972).

In two experiments, we implanted juvenile coho salmon with cortisol, using a molten (40°C) cocoa butter vehicle as described by Pickering and Duston (1983). Briefly, 4 mg of cortisol was dissolved in 1 mL of cocoa butter, and 0.1 mL of the mixture was injected intraperitioneally into each fish, resulting in a dose of about 0.01 mg of cortisol·g fish body weight $^{-1}$. This dose was previously found to elevate plasma cortisol to 60 ± 5 $ng \cdot mL^{-1}$ for up to 7 wk. Control fish received injections of cocoa butter without cortisol.

Experiments in which data were collected during time series were subjected to analysis of variance (ANOVA) and, when significant differences were found, Duncan's multiple range test (DMR test) for pairwise comparisons. Data collected at one time were analyzed by t-test. Percentages were arcsine transformed and subjected to chi-square anaylsis.

Experimental Designs and Results

Smoltification and Immune Response

In 1984, we sampled juvenile coho salmon from the Cote River stock biweekly from February 18 (mean fish weight, 32 g) to July 18 (mean fish weight, 63 g). Gill Na^+-K^+-ATPase activity and plasma T_4 in these fish peaked on about April 12 (Fig. 1A). After an early increase in plasma cortisol level, titers of this steroid increased significantly from initial values of about 5 to 25 $ng \cdot mL^{-1}$ during late May and early June (Fig. 1B). The number of PFC per million splenic lymphocytes was significantly depressed from April 12 to May 7, as compared with the three sampling dates before and after this period (Fig. 1B). There was also a significant decrease in circulating WBC relative to total circulating blood cells at the time when smoltification appeared to occur (Fig. 1C). Even though fish body weight increased during this time, there was no change in absolute number of splenic lymphocytes. However, this relation translated into a significant decrease in splenic lymphocytes per unit of body weight at the same time as the relative number of circulating WBC decreased (Fig. 1C).

In 1985 the same experimental design was used with coho salmon from Eagle Creek National Fish Hatchery. To determine if the kinetics of the immune response changed during smoltification, we measured splenic PFC from separate groups of fish 13, 17, and 21 d after immunization every 3 wk between February 19 (mean fish weight, 21 g) and June 4 (mean fish weight, 44 g). During this time, gill Na^+-K^+-ATPase activity and plasma T_4 and cortisol levels increased, peaking in late April to early May (Fig. 2). Moreover, there was no apparent change in the kinetics of the immune response during this developmental period, as numbers of PFC decreased independently of the number of days after immunization (Fig. 2B). We determined the sex of these fish based on gonad morphology and found no differences in response between sexes.

Rearing Conditions, Smoltification, and Immune Response

To study the effects of rearing conditions on immune function, we worked in association with Patino et al. (1987) who

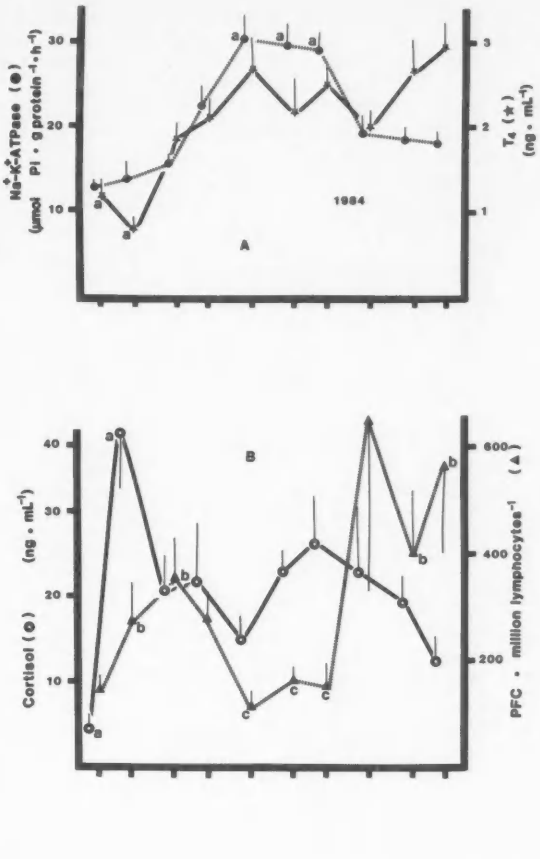

FIG. 1. Physiological and immunological measurements (mean + (−) 1 SE) from coho salmon, 1984. (A) Gill Na^+-K^+-ATPase activity and plasma T_4, n = 16−20; (B) plasma cortisol, n = 16−20, and number of plaque-forming cells (PFC), n = 8−12; (C) circulating white blood cells (WBC) as a percent of total circulating blood cells, n = 6, and number of splenic lymphocytes ($\times 10^7$) relative to fish body weight, n = 8−12. Points marked "a" are significantly different from all other points on the same line; points marked "b" are significantly different from points marked "c" on the same line ($p < 0.05$, Duncan's multiple range test).

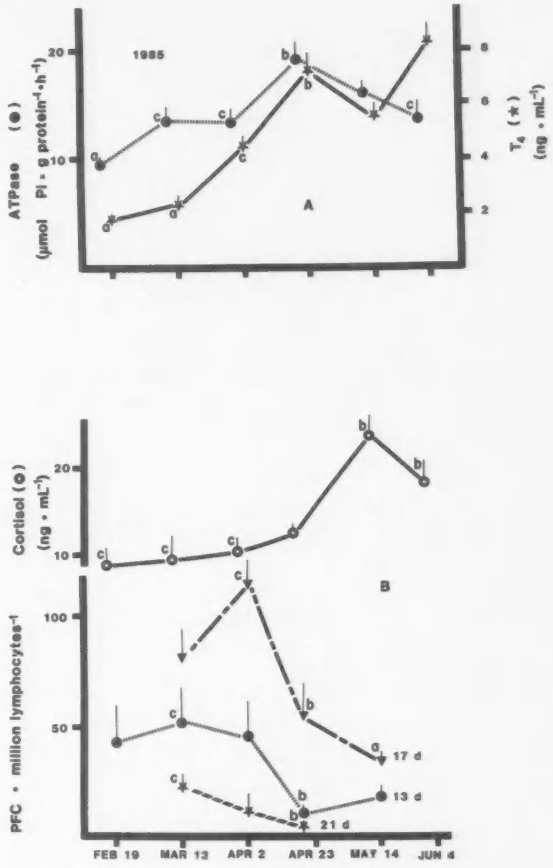

FIG. 2. Physiological and immunological variables (mean + 1 SE) from coho salmon, 1985. (A) Gill Na^+-K^+-ATPase activity and plasma T_4, n = 15−20; (B) plasma cortisol, n = 15−20, and numbers of plaque-forming cells (PFC), n = 8−10. Points marked "a" are significantly different from all other points on the same line; points marked "b" are significantly different from points marked "c" on the same line ($p < 0.05$, Duncan's multiple range test).

reported the effects of rearing density and water inflow rate on smoltification. Briefly, groups of Willard stock coho salmon were raised at high density (HD; 1941 fish·m^{-3}) or low density (LD; 647·m^{-3}) in flow-through circular tanks (0.9 m in diameter and 0.17 m^3 in volume). Fish at each density were exposed to two water inflow rates, 2.0 and 1.0 L·min^{-1}, for high inflow (HI) and low inflow (LI), respectively. Each treatment in this 2 × 2 design was duplicated. To examine the effect of short-term reduction of fish density on the indices of smoltification and immune function, we reduced (thinned) fish density in duplicated HD−LI groups to LD 2 wk before immunization. In June 1984, when the Willard stock is usually released from the hatchery, Patino et al. (1987) sampled fish for gill Na^+-K^+-ATPase activity, plasma T_4, and cortisol levels. Other fish from the same treatments were immunized with VOA, and PFC were determined 17 d later. The density at which fish were held affected all the variables tested; however, water inflow rate had no affect. Levels of the variables associated with smoltification were significantly lower in fish reared

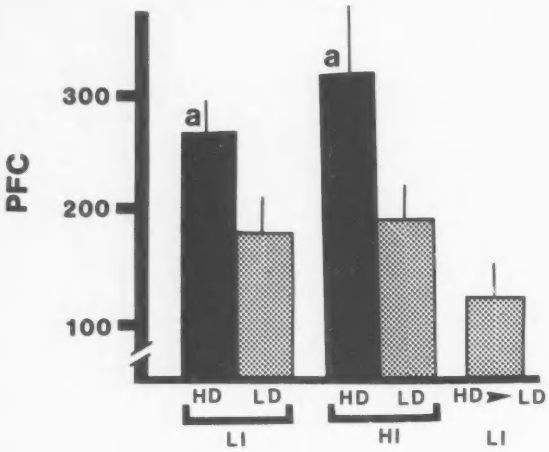

FIG. 3. Means (+1 SE) of plaque-forming cells (PFC) from coho salmon raised at high (HD) or low density (LD) and high (HI) or low water inflow rate (LI). Fish in two HD groups were thinned to LD (HD−LD) 2 wk prior to sampling. Bars marked "a" are significantly different from the others ($p < 0.01$, t-test).

at HD than at LD (T_4 approximately 1.5 versus 2.5 ng·mL^{-1}; cortisol, approximately 12 versus 20 ng·mL^{-1}; Na^+-K^+-ATPase, approximately 16 versus 24 μmol Pi·g protein^{-1}·h^{-1} Patino et al. 1987). Numbers of PFC were significantly greater in fish reared at HD than at LD (Fig. 3). When fish density was changed, the effects were mixed. Reducing fish density from HD to LD for 2 wk significantly increased plasma cortisol levels to about 20 ng·mL^{-1} with a concomitant decrease in PFC to about 118 per million lymphocytes (Fig. 3), but had no effect on gill Na^+-K^+-ATPase activity or plasma thyroxine titer (Patino et al. 1987).

Exogenous Cortisol and Immune System

Results of our experiments during smoltification and the fact that glucocorticoids have been shown to be immunosuppressive in fish (Anderson et al. 1982) suggested that the differences in PFC in response to VOA immunization might be linked to changes in plasma cortisol levels. To examine this link, groups of 24−26 juvenile coho salmon were acclimated to 64.5-L cuboidal aquaria that received a continous supply of aerated, fish-pathogen-free well water at 15.0 ± 0.5°C. Fish in one half of the groups received intraperitoneal injections of cortisol in cocoa butter and the rest received injections of cocoa butter (CB). Eleven days after injection, we exposed two groups of cortisol-implanted fish and two groups of CB-implanted fish to *V. anguillarum* (LS-174) using the waterborne infection method of Gould et al. (1978). An additional group from each treatment was treated similarly but was not exposed to the pathogen. Each disease-challenged group was exposed for 20 min to 1.5 million *V. anguillarum* cells·mL water^{-1} in 20 L. The bacterial cultures were grown at 18°C in brain−heart infusion broth. Culture purity was determined by bacterial shape, motility, sensitivity to vibrostat 0/129 (2,4-diamino-6,7-diisopropyl pteridine), and by rapid slide agglutination with *V. anguillarum* antisera. Dead fish were removed daily during the 14-d test. *Vibrio anguillarum* was isolated from kidneys of all dead fish. Plasma cortisol was assayed in fish treated with cortisol or CB that were not exposed to the pathogen. Plasma cortisol levels were about 10 times higher in cortisol-implanted fish than CB-implanted fish, and exposure to *V. anguillarum* resulted in significantly greater mortality in the cortisol-implanted than the CB-implanted groups (Table 1).

The effects of exogenous cortisol treatment on the number of PFC in response to VOA immunization and on the number and distribution of lymphocytes were also examined. We injected VOA into juvenile coho salmon from Eagle Creek National Fish Hatchery 7 d after cortisol or CB implantation, and 17 d later PFC were assayed in spleens and anterior kidneys. Kidney samples were collected in a way that ensured consistent sampling of tissue. Furthermore, one person dissected the kidneys from all fish without knowing the source of fish. Again, cortisol implants significantly elevated plasma cortisol levels, this time about 30-fold (Table 2). Mean cortisol level in the cortisol-implanted fish was only slightly lower than that of the peak seen in the same stock of fish during smoltification (Fig. 2). The immune system was also affected, as the cortisol-implanted fish had significantly fewer PFC in the spleens and anterior kidneys, fewer splenic lymphocytes, and fewer circulating WBC than did the CB-implanted fish (Table 2). Cortisol treatment also caused a shift in the relative composition of circulating WBC (Table 2).

Discussion

Our results have important implications relative to changes in immune competence and disease resistance of salmonids during smoltification and times of stress. Others have reported seasonal changes in the immune systems of fish, but these changes have been correlated to water temperature or sexual maturation (Yamaguchi et al. 1980; Wishkovsky and Avtalion 1982; Honma and Tamura 1984). We demonstrated that as coho salmon went through smoltification at a constant temperature, they became less able to generate a primary immune response to antigens of *V. anguillarum*, the causative agent of vibriosis (Fig. 1 and 2). The accompanying reduction in relative numbers of splenic lymphocytes and circulating WBC (Fig. 1C) suggests that general immune competence may have been impaired as well. This impairment occurred at a time when gill Na^+-K^+ -ATPase activity, plasma T_4 and cortisol levels were all elevated. Rearing conditions, such as rearing density, which appear to alter the rate of smoltification (Patino et al. 1987), also altered the ability of the fish to generate the immune response (Fig. 3). Short-term changes in rearing density that affected plasma cortisol levels, but not gill Na^+-K^+-ATPase activity or plasma T_4 levels, also altered immune response, suggesting that of the three physiological variables measured as indices of smoltification, only plasma cortisol affected the immune system. Implantation of exogenous cortisol resulted in essentially the same changes in the immune system (i.e. decreased numbers of PFC, splenic lymphocytes, and circulating WBC and reduced resistance to disease) as occurred during smoltification, again suggesting that this steroid plays a regulatory role in the fish immune system.

These findings are in agreement with those of McLeay (1975a), who found decreases in circulating WBC during stream residence of juvenile coho salmon, and suggested that these decreases were the result of adrenocorticosteroid activation during acclimation to cold water, and smoltification. McLeay (1973a, 1973b) also reported reductions in circulating WBC in juvenile coho salmon following treatment with exogenous glucocorticoid and adrenocorticotrophic hormones.

TABLE 1. Mortality data and plasma cortisol (±1 SE) of juvenile coho salmon exposed to *Vibrio anguillarum* (LS-174) 11 d after they received an intraperitoneal injection of 0.4 mg of cortisol in molten cocoa butter or cocoa butter.

Treatment replicates	Number of fish exposed	Mortalities (%)[a]	Mean time from exposure to death (d)	Mean plasma cortisol ($ng \cdot mL^{-1}$)[b]
Cortisol				
1	24	92	3.6	55.1±7.8
2	24	75		
Cocoa butter				
1	25	44	3.3	5.1±1.0
2	26	69		

[a]Treatments significantly different ($p < 0.01$, chi-square test).
[b]$n = 20$ fish; treatments significantly different ($p < 0.01$, *t*-test).

TABLE 2. Number of plaque-forming cells (PFC), distribution of lymphocytes, and cortisol titers in juvenile coho salmon immunized with *Vibrio anguillarum* O-antigen. Seven days before immunization, fish received implants of 0.4 mg of cortisol in cocoa butter or cocoa butter (CB). All values are mean ± 1 SE of eight fish, except for blood smears where sample size is four fish.

	Cortisol	CB
Plasma cortisol ($ng \cdot mL^{-1}$)	14.3±1.9[a]	0.5±0.4
Spleen		
Lymphocytes (million)	22.8±4.7[b]	45.5±9.4
PFC per spleen	77±55[a]	298±53
PFC per million lymphocytes	3±1[a]	7±1
Anterior kidney		
Lymphocytes (million)	19.0±2.2	20.0±1.0
PFC per anterior kidney	50±27[a]	135±11
PFC per million lymphocytes	4±3	7±1
Peripheral blood		
Red blood cells ($million \cdot mL^{-1}$)	1307±156	1218±75
White blood cells ($million \cdot mL^{-1}$)	70±10[b]	102±19
Differential cell counts (%)		
Large lymphocytes	1.4±0.5	1.8±0.7
Small lymphocytes	86.7±3.3[b]	78.0±5.0
Polymorphonucleocytes	4.9±1.2	9.8±3.7
Thrombocytes	6.9±2.1	10.4±1.8

[a]Significantly different from controls ($p < 0.01$, *t*-test).
[b]Significantly different from controls ($p < 0.025$, *t*-test or chi-squared test).

However, he found that the numbers of small lymphocytes were reduced in field studies (McLeay 1975a) and laboratory studies (McLeay 1973a, 1973b), whereas we found equal reductions of all WBC subpopulations during smoltification. These discrepancies may reflect differences in fish size or environmental factors between these studies.

Although we report changes in the immune system during smoltification, other investigators have reported that stress can depress the numbers of circulating WBC in fish (Pickford et al. 1971; McLeay 1975b; Tomasso et al. 1983), and work by Pickering and Duston (1983), Pickering (1984), and Pickering and Pottinger (1985) has shown a connection between stress-induced elevation of plasma cortisol and susceptibility to disease. Pickering and Duston (1983) and Pickering and Pottinger (1985) reported increased plasma cortisol levels and increased suceptibility to several bacterial pathogens present in the water source after brown trout (*Salmo trutta*) had been implanted with cortisol. However, Pickering and Pottinger (1985) did not find any effect of cortisol on numbers of circulating WBC. In our study, cortisol-implanted fish had reduced disease resistance, accompanied by reduced numbers of circulating WBC and splenic lymphocytes, but no reduction in the number of lymphocytes in the anterior kidneys. Moreover, the decrease in number of PFC per million lymphocytes in spleens and anterior kidneys of cortisol-implanted fish indicates that the observed immunosuppression was caused by mechanisms other than lympholysis alone.

Exogenous corticosteroid treatment in mammals can cause lympholysis, export of cells from thymus, spleen, and circulation, and sequestering of T-cells in bone marrow (Claman 1972; Cohen 1972; Fauci and Dale 1975). Although the B- and T-cell paradigm has not been verified for fish, there is considerable evidence of lymphocyte heterogeneity in fish (Etlinger et al. 1976; Cuchens and Clem 1977; Ruben et al. 1977) and that the anterior kidney in fish may be functionally similar to the bone marrow in mice (Zapata 1979; Kaattari and Irwin 1985). We report here that after long-term cortisol treatment of coho salmon, the numbers of antibody-producing cells in the spleen and anterior kidney were reduced; however, the kidney showed no reduction in lymphocyte number although splenic lymphocytes and circulating WBC were reduced. This relation suggests that antibody-producing cells were reduced in all organs but that another subpopulation of lymphocytes, perhaps T-like cells, was sequestered in the anterior kidney.

Although the functional significance of this apparent cortisol-mediated depression in immune competence during smoltification remains elusive, Munck et al. (1984) suggested that corticosteroids released during stress in mammals may function to prevent the immune system from generating autoimmunity and consequently damage to body tissues. Similarly, we speculate that if there are tissue changes during smoltification, cortisol may suppress immune function to avoid damaging tissues.

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Structure and Function of Phytoplanktonic and Microbial Communities in Relation to Industrial Wastewater Discharge: An Ecotoxicological Approach in a Lotic System

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Microbial and phytoplanktonic processes of a perturbed river system were evaluated as an ecotoxicological alternative to bioassays in hazard assessment of industrial wastewater discharge. Although phytoplankton species composition was invariable throughout the river gradient, both *P/B* and ATP/AMP ratios revealed that the community did not acclimate over the spatial scale studied. The adenylate energy charge (EC_A) of the microbial community decreased at the station where the wastewater is discharged; however, no significant differences in EC_A values were observed downstream. Enclosure experiments allowed us to conclude that the microbial community was predominantly affected by the effluent rather than by the lotic physical gradient. In the river gradient, EC_A appears to be an insensitive metabolic indicator; however, small changes in EC_A are accompanied by much larger changes in the ATP/AMP ratios to which enzymatic activities are responding. The fact that some functional metabolic processes of the microbial community were affected while phytoplankton species composition was invariable suggests that *P/B* and ATP/AMP ratios would prove useful in the detection of insidiuous effects of industrial effluents at the microbial community level. This approach could also give more insight into the ecological significance of wastewater discharge for monitoring purposes.

Diverses caractéristiques structurelles et fonctionnelles des communautés phytoplanctoniques et microbiennes d'une rivière polluée sont évaluées et proposées comme une alternative au dépistage des dangers environnementaux suite aux déversements d'effluents industriels. Bien que la structure de la communauté phytoplanctonique soit demeurée semblable le long du gradient de rivière, des variations significatives des rapports *P/B* et ATP/AMP montrent le manque d'acclimatation de la communauté à l'intérieur du tronçon de rivière étudié. La charge énergétique en nucléotides adénylates (CE_A) diminue à la station qui reçoit les eaux résiduaires toxiques; cependant, aucune différence significative ne persiste au niveau des trois sections situées en aval de l'égoût industriel. Des expériences réalisées en enclos nous ont permis de discriminer les effets de l'effluent par rapport à ceux du gradient physique de conditions rencontrées dans la rivière. Dans le tronçon de la rivière, la CE_A semble un indicateur métabolique moins sensible que le rapport ATP/AMP lequel est réputé contrôler certaines activités enzymatiques. Il apparaît donc que les rapports *P/B* et ATP/AMP soient utiles pour détecter des effets insidieux au niveau de la communauté microbienne alors que des effets toxiques directs (i.e. diminution de la biomasse, modification de la structure de la communauté) ne sont pas perceptibles. L'approche utilisée permet de plus d'obtenir une information fiable des effets écotoxicologiques d'effluents dans une perspective de surveillance de la qualité des eaux résiduaires.

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Short-term effects of pollutants on populations of organisms may cause changes in growth, reproduction, and/or metabolic functions. Long-term impacts may reveal major shifts in species composition and predator–prey relationships. At the microbial level of organization, the short time scale of reproduction adds another dimension to the problem: that of acclimation versus species replacement. It thus appears, as pointed out by Matthews et al. (1982), that information about both functional (metabolic) and structural (species composition, biomass) changes is necessary to the understanding of the response of an ecosystem to the impact of pollutants.

In such a context, we investigated both an anabolic indicator, the light-saturated rate of photosynthesis per unit chlorophyll (*P/B* ratio: micrograms carbon per microgram Chl *a* per hour), and an energy balance indicator, the adenylate energy charge ratio (EC_A: ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]), for the measurement of the physiological state of a natural microbial community (Atkinson 1968). The *P/B* ratio reflects the physiological state of the phytoplanktonic community whereas EC_A is related to the whole microplanktonic community (non-photoautotrophic and photoautotrophic populations; De Souza-Lima and Romano 1983).

Despite the fact that it is difficult to predict growth rate from short-term carbon assimilation in nutrient-limited experiments (Morris 1981; Harris 1984; Trainor 1984), there is evidence that this physiological sensor is sensitive to the effects of pollutants on indigenous phytoplankton populations (Rai et al.

1981). For example, Blinn et al. (1977) have shown a negative relationship between carbon assimilation and mercury concentrations after an exposure time of 2 h to the metal (0.013–0.109 mg Hg · L^{-1}). Other studies dealt with the relationship between carbon assimilation and biomass decreases. Wurtsbaugh and Horne (1982) conducted in vitro multiparameter bioassays on lake plankton; they found in a 6-d experiment that at copper concentrations above 20 μg · L^{-1} the inhibition patterns were similar for nitrogen and carbon assimilation as well as for Chl *a*. Côté (1983) has also conducted in vitro bioassays for assessing the copper toxicity on the standing crop and the photosynthetic process of phytoplankton from the Saguenay River (Québec); he found that at higher concentrations of copper (50–400 μg · L^{-1}), reductions in Chl *a* were low whereas rates of primary production were much more depressed. These results led us to use the P/B ratio to determine if acclimation was observed following the perturbation of an indigenous phytoplankton community; there is a lack of information on the sensitivity of this physiological sensor for assessing the effects of complex industrial wastewater discharges on the physiological response (acclimatory or non-acclimatory) of a phytoplankton community within a river system. Moreover, indigenous physical variables in lotic systems present a continuous gradient of physical conditions. This gradient induces within the constituent populations a series of responses resulting in a *continuum* of biotic adjustments (Vannote et al. 1980) which render problematic the discrimination between a pollutant's toxic effect and the effect of physical conditions on the indigenous populations of a river system.

EC_A is derived from the theory of adenylate control, formulated by Atkinson (1968). It is considered to be a linear measurement of the amount of energy momentarily stored in the nucleotide pool: the relative concentrations of adenosine triphosphate [ATP], adenosine diphosphate [ADP], and adenosine monophosphate [AMP] reflect the instantaneous metabolic energy available to a cell (Atkinson 1977). As reported by Fitzwater et al. (1983) and Heath (1984), the theoretical range of EC_A varies from 0.0 (all AMP) to 1.0 (all ATP); however, unstressed forms of invertebrates and microorganisms show EC_A values above about 0.8 (Atkinson 1977; Ivanovici 1980). Some studies indicate EC_A variations under altered conditions such as toxic chemicals, salinity, temperature, light, and nutrient starvation (Chapman et al. 1971; Ivanovici 1980; Fitzwater et al. 1983; Heath 1984). Walsh and Merrill (1984) have suggested the use of EC_A to determine end-points of algal toxicity tests for the detection of sublethal effects of complex wastes. These authors have expressed the need for more research to adapt EC_A as a tool for assessing the effects of exposure to complex wastes.

Our aim is to evaluate the P/B and EC_A ratios as in situ phytoplanktonic or microbial indicators for hazardous toxicant assessment and to test the hypothesis of the acclimatory response of a river community before and after the discharge of an industrial effluent. The hypothesis of the predominant effect of the industrial effluent over the lotic physical gradient on the microbial community is verified by the evaluation of the presence and persistence of effluent effects in enclosures.

Study Area

The Saint-Louis River (Fig. 1) is located on the south bank of the St. Lawrence River some 50 km southeast of Montréal (Québec). The river flow is parallel to the Beauharnois Canal in a southwest–northeast direction and empties into Lake Saint-Louis at the town of Beauharnois. Three towns, supporting agricultural activities, are located on the 145.5-km^2 drainage basin of the Saint-Louis River. The river flows on a bed of clay.

Certain physico-chemical characteristics of this river have been reported by Roy (1982) and Moulins (1983). Depth varies between 1.83 and 6.7 m, water transparency (Secchi) fluctuates between 0.4 and 2.5 m, and current speeds vary from 4.6 cm · s^{-1} during low-flow periods to 59.6 cm · s^{-1} during high-flow periods. As well as draining agricultural land, the river receives numerous industrial effluents (ferro-alloys, paper, chlorine, caustic soda, sodium chlorate, and aluminium). Cadmium, copper, chromium, iron, lead, manganese, mercury, nickel, and zinc loads constitute the main sources of pollution of the river. Moreover, the manganese, iron, and mercury concentrations remain abnormally high in the Lake Saint-Louis sediments (Roy 1982).

Materials and Methods

Enclosures

The experimental design consisted in exposing the upstream indigenous microbial community to various concentrations of industrial effluent and comparing the community response obtained with that of diluted controls. The experiment which lasted 22 h was carried out in situ in 12-L enclosures. A large volume of water (60 L) containing the indigenous microbial community (without grazers) was obtained by filtering (100-μm mesh) water taken at cross-section A (Fig. 1). The collected sample was subdivided in 12-L carboys and amended to obtain the following effluent concentrations: 0, 10, 25, and 50% (v:v, effluent: river water). Since the addition of effluent diluted the initial biomass, the control was diluted with Millipore "Q" process water. ^{14}C uptake was measured in separate 2.5-L bottles, filled to obtain identical effluent concentrations, and spiked with 50 μCi (1.85 MBq) of $NaH^{14}CO_3$. All enclosures were incubated directly in the river. Samples for the analyses described below were obtained at 0 (09:45 local time), 1, 2, 8, and 22 h. The dark period fell between 8 and 22h of exposure.

River Gradient

The effects of the industrial effluent were also tested on the indigenous microbial community downstream from the effluent discharge. The experimental program was carried out over two sampling periods. Four sites (A, B, C, D) located upstream and downstream from the industrial effluent were identified. Each cross-section was made up of three stations, the first located approximately 5 m from the east bank, the second in the middle of the channel, and the third approximately 5 m from the west bank. The west bank station at site B is located directly at the outfall of the industrial effluent (Fig. 1). For each cross-section, a composite sample was prepared for the physical, chemical, and microbial characteristics of the river water. Temperature and pH were measured immediately after preparing each composite sample. Then, subsamples were prepared for chemical analyses at the laboratory using standard preservation methods (Environment Canada 1979). Physico-chemical analyses and coliform counts (total and fecal) were

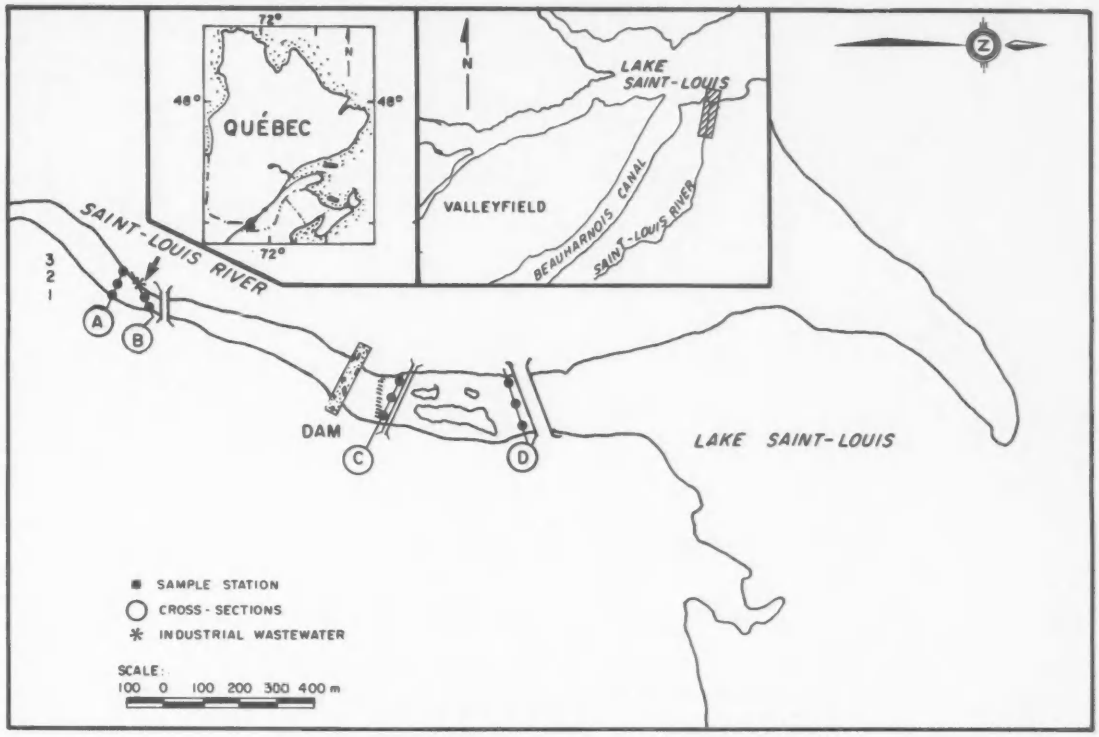

FIG. 1. Study area: identification of sampling stations.

performed according to standard procedures described in American Public Health Association et al. (1980). Phytoplankton samples were counted using Utermöl's method after preservation in an acidic lugol solution (Vollenweider 1974).

Biological Paramenters

Samples from the enclosure experiment and from each station were duplicated for determinations of Chl *a*, carbon assimilation, and adenylates content.

Chl *a* was determined after filtration of a 50-mL sample on a 0.4-μm polycarbonate filter (Nuclepore) and immediate extraction by placing the filters in 90% acetone (Strickland and Parsons 1972). Samples were kept in the dark for 2−4 d at 4°C and fluorescence was measured before and after acidification on a Turner Designs filter fluorometer. Extraction of photosynthetic pigments was used instead of in vivo determination because in vivo measurements are subject to interference from humic material (Carlson and Shapiro 1981).

For the river gradient experiment, ^{14}C uptake was determined with duplicate 300-mL subsamples spiked with 10 μCi (370 kBq) of radioactive $NaH^{14}CO_3$ and incubated for 1 h at light saturation in the river. A 20-mL subsample was then filtered on Nuclepore 0.4-μm membrane filters, whereas for the enclosure experiment duplicate 50-mL samples were filtered and radioactivity was determined using a model 1215 Rackbeta liquid scintillation counter (LKB Wallac). Samples were quench corrected using an hexadecane internal standard and the Sample Channels Ratio Method. The specific activity in the bottles was determined by injecting 100 μL into ethanolamine and subsequent counting in Scintiverse. Time zero blanks were corrected and ^{12}C assimilation was calculated from alkalinity and pH (Vollenweider 1974).

For adenylate nucleotide determinations, duplicate 100-mL samples were immediately filtered on 0.4-μm Nuclepore filters and immediately extracted by placing them in 4.5 mL of dimethyl sulfoxide (DMSO) followed by vortex mixing (40 s). Concentrated DMSO was used because of its recognized efficiency for adenylate extraction and because it is known to inhibit enzymes that hydrolyze ATP (Jakubczak and Leclerc 1980). Subsequently, 5.5 mL of a 10 m*M* 4-morpholinepropanesulfonic acid buffer (MOPS, pH 7.4) was added (Jakubczak and Leclerc 1980). The extracts were measured using a luminescence apparatus (LKB 1219 luminometer) and ultrapure enzymes obtained from LUMAC (St. Paul, MN). Briefly, three reagents labelled A, B, and C are utilized for determinations of ATP, ADP, and AMP, respectively. Reagent A is a 25 m*M* Hepes buffer containing 10 m*M* KCl at pH = 7.7. Reagent B is a mixture containing lyophilized phosphoenolpyruvate and pyruvate kinase which is reconstituted with 3.4 mL of reagent A. Reagent C contains the adenylate kinase in a 3.2 *M* $(NH_4)_2SO_4$ solution; prior to use, 1.7 mL of reagent B is mixed with 40 μL of reagent C. Finally, a fourth vial ("lumit PM") contains purified, lyophilized luciferine–luciferase extract which is reconstituted before use with 7 mL of "lumit" buffer. Nucleotide analysis was performed in duplicate as followed. In all cases, 50-μL aliquots of the unknown was mixed with 50 μL either reagent A (ATP), reagent B (ADP), or reagent C (AMP). Replicate samples were spiked with known amounts of nucleotide used as internal standards.

TABLE 1. Physical, chemical, and microbiological characteristics of the effluent and of river surface water.

Date (1984)	Cross-section	Temp. (°C)	pH	Solids		$[P_t]$	[Hg]	Coliform count (per 100 mL)	
				Total ($mg \cdot L^{-1}$)	Suspended ($mg \cdot L^{-1}$)	($mg \cdot L^{-1}$)	($mg \cdot L^{-1}$)	Fecal	Total
Sept. 26	Effluent		9.1	502	13.1	<0.01	0.0030	<3	<3
	River (A)	18.0	7.2	256	35.6	0.06	<0.0001	24 000	46 000
Sept. 27	A	14.0	8.1	255	30.7	0.12	<0.0001	24 000	46 000
	B	15.0	8.8	346	25.1	0.02	0.0010	430	930
	C	15.3	8.4	508	29.9	0.08	0.0009	930	930
	D	16.0	8.4	494	32.4	0.04	0.0007	9 300	21 000
Oct. 4	A	8.8	7.8	270	23.9	0.08	<0.0001	2 300	9 300
	B	10.1	8.7	323	16.3	0.04	0.0013	4 300	7 500
	C	11.4	8.2	293	19.6	0.06	0.0007	4 300	9 300
	D	11.6	8.0	295	21.5	0.08	0.0007	4 600	9 300
Oct. 5	A	8.9	7.9	278	16.2	0.04	<0.0001	4 300	46 000
	B	10.1	8.7	315	16.8	0.04	0.0012	2 300	23 000
	C	10.1	8.4	293	14.4	0.04	0.0006	4 300	43 000
	D	10.0	8.2	291	18.9	0.04	0.0007	4 300	24 000

TABLE 2. Taxonomic composition, relative abundance (%), and diversity of the phytoplanktonic community.

Date	Taxa	River cross-section				Enclosure experiment
		A	B	C	D	
Sept. 27	*Asterionella*	—	—	—	1	—
	Cocconeis	—	—	1	48	2
	Cyclotella	36	31	31	48	37
	Gomphonema	1	—	—	—	—
	Gyrosigma	1	—	—	—	—
	Melosira	9	12	5	8	7
	Navicula	5	2	2	4	2
	Nitzschia	4	—	1	1	6
	Total Bacillariophyceae	58	47	40	63	54
	Total Chlorophyceae[a]	38	49	52	33	46
	Total Cyanophyceae	4	4	8	4	—
	H'[b]	0.88	0.86	0.99	0.77	0.83
Oct. 4	*Cocconeis*	—	—	1	—	
	Cyclotella	28	30	26	22	
	Gyrosigma	—	—	2	—	
	Melosira	8	6	17	13	
	Navicula	1	4	13	4	
	Nitzschia	1	—	3	2	
	Stephanodiscus	—	—	1	—	
	Total Bacillariophyceae	45	42	74	46	
	Total Chlorophyceae	36	49	14	39	
	Total Cyanophyceae	19	9	12	15	
	H'	1.09	0.92	0.94	1.03	

[a]See text for composition.

[b]$H' = -\sum_{i=1}^{s} p_i \log p_i$ (Shannon–Weaver diversity index) where p_i is the proportion of the ith species in the population (Pielou 1966).

Incubation at room temperature lasted 30 min for ATP and ADP and 2 h at 30°C for AMP. Preliminary experiments (Madariaga 1986) have shown that the percent conversion AMP to ATP reaches a maximum after 2 h. In the case of the ADP determination, 100% conversion to ATP was obtained after 30 min. The preparation for the luminometric measurements involved filling of the dispenser tube with lumit PM extract, and loading of the sample tubes in the rotary sampler of the LKB luminometer. For ATP and ADP measurements, 100 µL of the enzyme solution was dispensed, while AMP determinations required 150 µL. Light emission increased rapidly and reached a plateau after 2 s; therefore, integrated or peak measurements yielded identical values. EC_A was calculated according to Atkinson's relationship described

previously. Using the procedure outline above, the detection limit for the three nucleotides was 0.25 n*M* for ATP and 0.5 n*M* for ADP and AMP; the coefficient of variation of the 95% confidence interval (2 × standard for deviation/mean × 100) at the 5 n*M* level was 5.7% (ATP), 6.9% (ADP), and 13.8% (AMP). Analyses of variance were carried out and an a posteriori comparison of means examined with Sheffe's test.

Results and Discussion

Physical and Chemical Characteristics

The important metal contamination of this river was demonstrated by Roy (1982) where it was clear that the lead, chromium, magnesium, copper, and mercury concentrations in the aquatic vegetation exceeded the average level of those elements in the macrophytes of the St. Lawrence River. Fedida (1982) determined the heavy metal concentrations of the effluent at various times over a 4-d period; concentrations (milligrams per litre) were as follows: Fe 0.101−0.426; Cr ≤ 0.020-0.192; Cu ≤ 0.020; Ni ≤ 0.010−0.014; Pb ≤ 0.010−0.015; Cd ≤ 0.010; Zn ≤ 0.010−0.058; Co ≤ 0.025; Hg 0.0023−0.0064. Total solids varied between 530 and 900 mg · L^{-1}, that of dissolved solids between 28 and 85 mg · L^{-1}, pH between 6.6 and 11.3, and temperature between 11.5 and 20.0° C. The toxicity of this industrial effluent to *Catostomus commersoni* and *Daphnia magna*, two indigenous species, was clearly shown by in situ bioassays (Moulins 1983). Between the effluent outfall and the mouth of the river in Lake Saint-Louis, the observed mortalities varied respectively between 97.5 and 6.8% (*C. commersoni)* and 100 and 1.7% (*D. magna)*.

Our results (Table 1) concerning the effluent are generally in agreement with those reported in the previous paragraph and clearly show its influence on temperature, pH, total solids, and total mercury observed at cross-section B. Despite effluent discharges, the Saint-Louis River remains eutrophic, since it drains numerous agricultural territories. The high inorganic phosphorus concentrations as well as an excess of coliforms in the river are indicative of this influence.

Phytoplanktonic Community Structure

The taxonomic composition of the phytoplanktonic community reflects the eutrophic state of this river. During the three sampling days, Bacillariophyceae made up 40−74% of the community and *Cyclotella* sp. was dominant at all four cross-sections (22−48% of the community). Various Chlorophyceae (*Sphaerocystis* sp., *Tetraedon* sp., *Scenedesmus* sp., *Ankistrodesmus* sp., *Oocystis* sp., *Tetraspora* sp., *Chrysococcus* sp., *Crucigenia* sp.) constituted 14−52% of the community (Table 2). Symoens *et al.* (1981) observed eutrophic environments dominated by diatoms, where *Melosira granulata* and *Cyclotella stelligera* were the most abundant species. During the summer months, such diatoms as *Tabellaria fenestrata, T. flocculosa, Asterionella formosa, Melosira* sp., and *Fragilaria* sp. are also dominant in the Saguenay River, a water system highly contaminated by heavy metals (Loring 1975, 1976; Smith and Loring 1981; Côté 1983).

The effluent discharge at cross-section B only causes a slight modification of the community structure. For example, on October 4, although *Cyclotella* sp. remained dominant, the diversity index H' (Pielou 1966) varied from a value of 1.09 bitt · $cell^{-1}$ at cross-section A to 0.92 bitt · $cell^{-1}$ at cross-section B. Diversity increased again at cross-section D, where $H' = 1.03$ bitt · $cell^{-1}$ (Table 2). In September, diatoms accounted for 58% of the community at A and 63% at D, whereas in October they represented 45% of the community at A and 46% at D. The most apparent modifications appear at C which is located immediately downstream from a dam. It should be pointed out that the flow at the dam outfall is high (Moulins 1983). Current speed renders unlikely the appearance of species modifications induced by the discharge of the industrial effluent, since the distance between cross-sections A and D would be traveled in either 0.5 h during high flow or 6.6 h during low flow (Moulins 1983). Thus, in the study area, the effects of the industrial effluent would be felt for a rather short period of time, making it unlikely that new species appear or become dominant. Harris (1984) has suggested that changes in algal species occur over time scales similar to those required for physiological adaptation, presumably >12−200 h. This stated time requirement for physiological adaptation, however, is much longer than is generally accepted for cellular responses to reductions in ATP, total adenylates (A_T), and energy charge (EC_A) values. Atkinson(1977) has put forward a time scale of <10 min for such response.

Functional Characteristic: *P/B* Ratios

The two sampling periods of October 4 and 5 revealed decreases of 70−91% between the mean *P/B* ratios in cross-section A (*P/B*= 0.64−0.95 and 0.23−0.48) which is not influenced by the discharge and those of cross-sections B (*P/B* = 0.10−0.36 and 0.04−0.07), C (*P/B* = 0.24−0.28 and 0.03−0.04), and D (*P/B* = 0.14−0.26 and 0.04−0.13) which are located downstream from the effluent outfall. Although *P/B* ratios vary widely from day to day according to variable environmental conditions, their response to the industrial effluent is, however, always highly significant (Table 3). The comparison of means examined with Sheffe's test shows that cross-section A was significantly different from cross-sections B, C, and D.

Rates of photosynthesis dropped from a mean value of 2.6 μg $C \cdot L^{-1} \cdot h^{-1}$ at cross-section A to mean values of 0.77, 0.83, and 0.69 μg $C \cdot L^{-1} \cdot h^{-1}$ at cross-section B, C, and D, respectively, with a low of 0.15 μg $C \cdot L^{-1} \cdot h^{-1}$ directly at the mouth of the effluent outfall. These low values are particularly significant when the Chl *a* data are considered. The industrial effluent discharge itself produced a temporary reduction in biomass at station 3B, where Chl *a* concentrations of 1.49 and 1.56 μg · L^{-1} were observed on October 4 and 5, respectively. This transitory decrease probably results from dilution by the effluent discharge, since in comparison, Chl *a* values generally remained above 3 μg · L^{-1} at all other stations on both sampling dates. The relative consistency of the Chl *a* values shows that the decreases in *P/B* ratios are related to decreases of the photosynthetic rates of the phytoplanktonic community.

The sensitivity of *P/B* ratios to toxic chemicals was also noted by Côté (1983) who recorded decreases >75% of the *P/B* ratios of Saguenay River phytoplankton assemblages submitted in vitro to high copper concentrations (>10 μg $Cu \cdot L^{-1}$). At these copper concentrations, *P/B* ratios never exceeded 0.1, while *P/B* ratios in the control never exceeded 0.4. The *P/B* ratios determined in the Saguenay River were lowest in May (0.1) and highest in August (2.1) (Côté 1983).

This is in agreement with Platt and Jassby (1976) who found a highly significant correlation of *P/B* ratios versus temperature ($N = 52$; $\alpha \leq 0.01$; $r = 0.529$). Platt et al. (1980) have also determined lower *P/B* ratios of 0.98 and 0.64 for stations

TABLE 3. Analysis of variance of P/B (production/biomass) ratio, EC_A (adenylate energy charge), and ATP/AMP ratio data collected on the Saint-Louis River on October 4 and 5, 1984.

Date	Source	df	Sum of squares	Mean squares	F-statistic	Probability
			P/B ratio			
Oct. 4	Effluent	3	0.784	0.261	18.1	0.001
	Residual	8	0.111	0.014		
	Total	11	0.895			
Oct. 5	Effluent	3	0.240	0.080	16.1	0.001
	Residual	8	0.039	0.005		
	Total	11	0.279			
			EC_A			
Oct. 4	Effluent	3	0.013	0.004	1.8	ns
	Residual	8	0.018	0.002		
	Total	11	0.031			
Oct. 5	Effluent	3	0.018	0.006	2.6	ns
	Residual	8	0.018	0.002		
	Total	11	0.036			
			ATP/AMP ratio			
Oct. 4	Effluent	3	1.84	0.61	2.77	$0.05 < \alpha < 0.10$
	Residual	20	4.33	0.22		
	Total	23	6.17			
Oct. 5	Effluent	3	2.08	0.69	7.19	$0.001 < \alpha < 0.005$
	Residual	20	1.92	0.096		
	Total	23	4.00			

located in the Arctic (T = 0.5 and −0.5°C); maximum values (15.2) are attained at much higher temperature (~19° C). The fact that P/B ratios remain low at all three cross-sections downstream from the effluent discharge indicates that during the time scale considered (between 0.5 and 6.6 h), the photosynthetic activity of the phytoplanktonic community does not become acclimated to the toxic effluent.

The sum of the nucleotides (A_T) decreases as the microbial community reaches the industrial effluent discharge. A_T values of the order of 1.38−1.62 nM and 1.81−2.01 nM were observed on October 4 and 5, respectively, in cross-section A samples, in comparison with values of 0.84−1.36 nM and 0.80−1.71 nM at cross-section B (directly at the effluent outfall). At cross-sections C and D, A_T values remained lower than those measured in the cross-section A samples of October 5, while higher concentrations (1.53 −2.06 nM) were observed on October 4. These variations in the nucleotide pool are probably the result of dilution by the effluent, since relatively constant Chl a/A_T ratios (0.0047 ± 0.0009) were observed at all cross-sections on both sampling dates, indicating that both biomass indicators show proportional variation on dilution.

Functional Characteristic: EC_A

Studies have shown that activities of a number of enzymes are related to the concentration of ATP, some to ADP or AMP while others are dependent on the ATP/AMP or the ATP/ADP ratios. For example, metabolic energy transduction is built around the maintenance of the ATP/ADP ratio (Chapman and Atkinson 1977), while it appears that a low ATP/AMP ratio stimulates glycolysis and a high ATP/AMP ratio activates gluconeogenesis (Atkinson 1977). The cumulative effects of the adenosine phosphates on the rate of cellular metabolism are expressed in terms of the EC_A parameter (Karl 1980).

In the present study, EC_A values varied between 0.58 and 0.68 and between 0.62 and 0.64 on October 4 and 5, respectively, at the three stations above the outfall (cross-section A). These values are slightly lower than those previously suggested for nonstressed microorganisms and are indicative of a phytoplanktonic population in stationary phase (Falkowski 1977).

At the effluent discharge, a drop in EC_A values is observed. For example, during the two sampling periods, decreases averaged 12 and 16% at cross-section B. The most noticeable decrease (29%) occurred at the station closest to the effluent outfall (station 3 B) where EC_A values of 0.48 and 0.44 were measured on October 4 and 5, respectively. Further downstream (cross-sections C and D) the EC_A values remained lower than those observed at cross-section A: they were generally 10−15% lower. Although these results are in agreement with the suggestion that lower EC_A values indicate the presence of stressed microorganisms (De Souza-Lima and Romano 1983), the differences in EC_A values observed between upstream (cross-section A) and downstream stations (cross-sections B, C, and D) are not significant (Table 3). However, since an EC_A of approximately 0.5 has been suggested to be the value critical to the maintenace of viability (Falkowski 1977), the reductions observed at the cross-sections downstream from the industrial effluent discharge appear significant from a physiological standpoint. EC_A values such as 0.44 and 0.48 (station 3B),

TABLE 4. Variation of the ATP/ADP and ATP/AMP ratios of the microbial community before (cross-section A) and after (cross-sections B, C, D) the effluent discharge for the sampling period of October. Asterisks indicate outliers.

	Cross-section			
	A	B	C	D
ATP/ADP 1	3.71	5.39	3.65	4.75
	3.71	*	5.11	3.84
2	1.96	2.81	3.16	8.22
	2.33	*	4.21	*
3	4.03	3.31	4.48	6.05
	4.52	4.04	5.30	7.27
Mean ± SD	3.38 ± 1.01	3.89 ± 1.12	4.32 ± 0.83	6.03 ± 1.78
ATP/AMP 1	1.66	1.36	1.25	0.99
	2.37	1.35	1.40	1.12
2	1.80	1.66	1.33	1.31
	2.30	1.27	1.14	1.35
3	1.74	0.65	1.20	1.33
	1.70	0.83	1.18	2.05
Mean ± SD	1.93 ± 0.32	1.19 ± 0.37	1.25 ± 0.10	1.36 ± 0.37

0.51 (station 1D), 0.52 (stations 2C and 3C, station 1D), and 0.53 (station 2D) suggest that the viability of the microbial community is endangered. However, as pointed out by Karl (1980), the strict interpretation of community EC_A is difficult, since the community may be composed of active and inactive cell populations. The initial reduction in the EC_A reveals either a greater intracellular energy demand not fully compensated or, alternatively, a rapid change in the adenylate ratios by direct blockage of ATP yielding pathways such as electron-transfer phosphorylation and/or glycolysis in the microbial community. Since in most algae, photophosphorylation is the primary ATP-yielding pathway (Raven and Beardall 1981), it appears more likely that the inhibition of photosynthesis rather than of glycolysis is responsible for the reduction in EC_A.

A drop in EC_A results from the accumulation of AMP when there is a sudden increase in energy demand on the adenine nucleotide pool (Chapman and Atkinson 1977; Fitzwater et al. 1983; Heath 1984). When the $ATP \rightarrow AMP + P_i \sim P_i$ reaction is favored over the $ATP \rightarrow ADP + P_i$, direct pyrophosphate cleavage is accompanied by greater energy liberation. This type of reaction is encountered in the enzymatic activation of fatty acids, in the form of esters CoA. They are an important group of activated intermediaries in the biosynthesis of lipids and some amino acids (Lehninger 1977). It is conceivable that the cell could derive the energy required to respond to stressful conditions by an analogous reaction ($ATP \rightarrow AMP + P_i \sim P_i$). This will lead to a decrease in the ATP/AMP ratio. The removal of the accumulated AMP would tend to restore high energy charge values. In view of the reaction $2ADP \leftrightarrows ATP + AMP$ catalyzed by adenylate kinase, it is obvious that the removal of AMP will cause the conversion of ADP to ATP and thus tend to increase the ATP/ADP ratio and the EC_A. Removal of AMP would be effected by its cleavage to adenine and ribose 5-phosphate, catalyzed by adenosine monophosphate nucleosidase. The rate of this enzyme reaction is known to increase sharply as the EC_A decreases from its normal value. Alternatively, AMP can be lost by excretion into the growth medium (Chapman and Atkinson 1977).

On October 5 particularly, our results at cross-section B show a significant reduction in the ATP/AMP ratio which is concurrent with the observed drop in EC_A (Tables 3 and 4). Although the ATP/AMP ratio increases at cross-sections C and D, it is not restored to original levels over the time scale studied. Scheffe's test shows that the ATP/AMP ratios at cross-section A are significantly higher than at cross-sections B, C, and D. Downstream from the effluent discharge (cross-sections C and D) an increase in the EC_A is noted and, as can be expected, it is accompanied by a marked increase in the ATP/ADP ratio at cross-section D (Table 4).

Enclosure Experiment

The enclosure experiment was carried out using the phytoplanktonic community collected at cross-section A. Its composition is very similar to the one encountered during the river gradient experiment (Table 2). In the control enclosure, except for the first hours of the experiment, Chl *a* remained relatively constant and after 22 h reached a value $4 \cdot 6\ \mu g \cdot L^{-1}$. On the other hand, total adenylates (A_T), comprising microbial and phytoplanktonic biomass, increased from 2.43 to 4.45 n*M* during the 22 h of the experiment. The Chl a/A_T ratio varied from 1.81 at time 0.1 h to 1.03 after 22 h, possibly indicating a greater increase in bacterial biomass. The influence of the industrial effluent on Chl *a* and A_T is apparent at all concentrations used. At the end of the experiment, inhibitions of 78−89% and 68−75% were observed for Chl *a* and A_T respectively.

Photosynthesis is greatly reduced at all effluent concentrations used. At the end of the experiment, carbon assimilation by the phytoplanktonic communities in 10, 25, and 50% effluent was 17, 4, and 2%, respectively, of the control. The *P/B* ratios of the control enclosure, which varied between 3.01 and 2.07 in the first 8 h of the experiment, are much higher than those measured at cross-section A of the Saint-Louis River. This is possible due to the absence of turbulence in the enclosure. As was the case in the river cross-section (B) receiving the effluent discharge, there is a highly significant reduction in the photosynthetic efficiency of the microbial community in the effluent-treated enclosures (Table 5). For

TABLE 5. Analysis of variance on P/B ratios, EC_A, and ATP/AMP and ATP/ADP ratios of the enclosure experiment.

Parameter	Source	df	Sum of squares	Mean square	F-statistic	Probability
P/B ratio	Effluent	3	28.06	9.35	34.63	0.001
	Residual	20	5.32	0.27		
	Total	23	33.38			
EC_A	Effluent	3	1.40	0.47	58.75	0.001
	Residual	28	0.21	0.008		
	Total	31	1.61			
ATP/AMP ratio	Effluent	3	16.51	5.5	78.57	0.001
	Residual	28	1.83	0.07		
	Total	31	18.34			
ATP/ADP ratio	Effluent	3	175.15	58.38	24.53	0.001
	Residual	28	66.67	2.38		
	Total	31	241.82			

TABLE 6. Temporal variations of EC_A and ATP/ADP and ATP/AMP ratios of the microbial community in control and effluent-treated enclosures. Values are replicated.

	Effluent concn. (%)	Exposure time (h) 0.1	1	2	8	22
EC_A	Control	0.61	0.64	0.65	0.62	0.66
		0.59	0.63	0.67	0.47	0.67
	10	0.49	0.46	0.44	0.29	0.26
		0.50	0.48	0.45	0.36	0.24
	25	0.43	0.28	0.23	0.10	0.07
		0.39	0.31	0.21	0.09	0.08
	50	0.36	0.18	0.11	0.05	<0.01
		0.33	0.17	0.10	0.04	<0.01
ATP/ADP	Control	3.54	9.38	4.83	6.34	3.55
		8.47	5.86	4.19	5.26	9.13
	10	4.50	7.00	2.50	1.61	1.33
		4.39	4.23	4.81	1.11	1.47
	25	3.20	1.44	1.09	0.25	0.07
		3.79	1.11	1.00	0.16	0.06
	50	1.55	0.42	0.20	<0.01	<0.01
		1.61	0.46	0.15	<0.01	<0.01
ATP/AMP	Control	1.72	1.90	2.04	1.31	2.17
		1.48	1.79	2.25	1.16	2.16
	10	0.97	0.85	0.74	0.40	0.28
		0.98	0.90	0.79	0.40	0.27
	25	0.63	0.36	0.21	0.04	0.01
		0.69	0.32	0.20	0.02	0.01
	50	0.44	0.11	0.04	<0.01	<0.01
		0.46	0.11	0.03	<0.01	<0.01

example, P/B ratios varied between 3.01 and 2.07 in the control enclosure in comparison with values below 0.4 in 25 and 50% effluent.

Except at 0.1 and 8 h, the EC_A of the control enclosure was maintained above 0.6 (Table 6). However, in the effluent enclosures, EC_A decreased sharply and was maintained below the critical 0.5 viability level (Falkowski 1977). In 25 and 50% effluent, EC_A was below 0.1 for the last 14 h of the experiment. The lowered EC_A values result from an increase in AMP which is reflected in lowered ATP/AMP ratios. This is in fact observed in the effluent-treated enclosures where the ATP/AMP ratios vary from 0.98 to 0.27 in 10% effluent, from 0.69 to 0.01 in 25% effluent, and from 0.44 to <0.01 in 50% effluent compared with variations from 1.48 to 2.17 in the control (Table 6).

A one-way ANOVA carried out on the adenylate ratio data revealed that the differences observed in EC_A and ATP/AMP and ATP/ADP ratios between the control and effluent-treated enclosures are highly significant (Table 5).

From those results, it is obvious that the industrial effluent is toxic for the microbial community of the Saint-Louis River. The toxicity is reflected by the observed reductions of both structural (Chl a and A_T) and functional (photosynthesis, EC_A, and ATP/AMP ratio) characteristics of the community. In this light, the decreases in photosynthetic efficiency, EC_A, and ATP/AMP ratios observed in transects B, C, and D of the Saint-Louis River can be attributed to the discharge of effluent at cross-section B. Over the time scale studid (0.5 – 6.6 h in the river gradient experiment; 22 h in the enclosure experiment), acclimation to the effluent is not observed suggesting irreversible effects. Enclosure experiments allowed us to conclude that the microbial community was predominantly effected by the effluent rather than by the lotic physical gradient.

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Effect of Changes in Zooplankton on Orthophosphate Dynamics of Natural Phytoplankton Communities[1]

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McCauley, E., and J. Kalff. 1987. Effect of changes in zooplankton on orthophosphate dynamics of natural phytoplankton communities. Can. J. Fish. Aquat. Sci. 44: 176–182.

Replicate algal communities were isolated from Lake Memphremagog (Quebec–Vermont) in large–volume enclosures, and the abundance of herbivores was changed in them to produce a gradient of herbivore biomass among enclosures. The performance of the phytoplankton and their phosphorus uptake was observed in response to these different levels of crustacean zooplankton biomass. In general, reducing zooplankton biomass did not lead to phosphorus-deficient algal communities. Seven field experiments were done in 1979, 1980, and 1981. Only two of these experiments produced algal communities in which the cycling rate of orthophosphate per unit algal biomass was modified by reduction of crustacean zooplankton biomass.

Dans le lac Memphremagog (Québec – Vermont), on a isolé des communautés algales en plusieurs exemplaires dans de grands enclos et on y a modifié le nombre d'herbivores afin d'obtenir un gradient de biomasse entre les enclos. Le rendement du phytoplancton et sa capacité de captation du phosphore ont été observés en fonction des différentes biomasses de crustacés zooplanctoniques. En général, une réduction de la biomasse zooplanctonique n'a pas donné lieu à une carence en phosphore des communautés algales. Des sept expériences menées sur le terrain en 1979, 1980 et 1981, seules deux ont produit des communautés algales où le taux de transport cyclique de l'orthophosphate par unité de biomasse algale a été modifié par la baisse de la biomasse de crustacés zooplanctoniques.

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In the extreme, two interpretations of factors governing phytoplankton community structure and dynamics can be contrasted. One view is that phytoplankton are in equilibrium with their resources; their average density is set by the average availability of nutrients in the system, and differences among species in their growth rates around this equilibrium can be used to predict changes in algal community structure. An alternative view is that algal populations are far from any equilibrium, and that their growth rates are density independent for most periods. Changes in community structure would, consequently, be related to differences among species in their intrinsic growth rates.

These extremes also affect views of phytoplankton–zooplankton interactions in lakes. Zooplankton eat phytoplankton and they regenerate nutrients essential for the growth of phytoplankton. However, these activities will have different effects on algal dynamics depending on the relation between the algal community and their resources. If algal populations are at a much lower level than could be supported by the available nutrients, then regeneration of nutrients by zooplankton will be unimportant, since algal growth rates will be density–independent. The primary effect of herbivores, under these conditions, will be as agents of mortality. But, when algal growth rates become nutrient dependent, the effects of herbivores are no longer decoupled, and predicting their impact on algal dynamics is much more complicated.

Under conditions of both nutrient sufficiency and deficiency, reducing zooplankton levels should lead to an increase in algal biomass. This increase should reduce the level of available nutrients and, depending on the severity of nutrient limitation, result in a decrease in algal growth rates. If no decrease in algal growth rates is observed, then one possible conclusion is that growth rates of the phytoplankton are not nutrient limited.

While it is common to observe an increase in algal biomass in field experiments where zooplankton biomass is reduced (e.g. Porter 1973; Briand and McCauley 1978; McCauley and Briand 1979; Lynch and Shapiro 1981; Lehman and Scavia 1985; McCauley and Kalff 1987), demonstrations of corresponding effects on nutrient dynamics or algal growth rates are equivocal. Lehman (1978, 1980) observed that increasing zooplankton abundance in enclosures sometimes increased dissolved nutrients (Lehman 1978: Lake Washington), while in other lakes it did not (Lehman 1978; Egg Lake). Based on an assumed ratio between oxygen consumption and phosphorus (P) release, Devol (1979) calculated P regeneration rates from respiration rates and concluded that zooplankton could supply a significant amount of P to Lake Washington phytoplankton during summer stratification.

To test further the importance of zooplankton in affecting the relationship between phytoplankton and their resources, we did a series of seven field experiments to examine variation in

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short-term dynamics of orthophosphate and algal biomass in response to reduced zooplankton biomass. Phosphorus was chosen because it is the primary limiting nutrient in most unpolluted lakes (Likens 1972; Schindler 1974), and orthophosphate is the form excreted by zooplankton (Peters and Rigler 1973; Peters and Lean 1973) that is directly available to the phytoplankton. The short-term dynamics of orthophosphate were observed by estimating the rate constant for orthophosphate uptake and the turnover time using radiobiological techniques (Rigler 1956; Lean and Rigler 1974; Peters 1975).

Phosphorus turnover time has been proposed as an index of P demand because it is a biologically controlled process that depends on the orthophosphate concentration, plankton biomass, and their cellular content (Rigler 1964; Lean and Nalewajko 1976, 1979; Norman and Sager 1978; Lean and Pick 1981). If phytoplankton biomass increases, then k will increase (Faust and Correll 1976; White et al. 1982), indicating an increase in demand for orthophosphate. Alternatively, if orthophosphate concentration decreases, then k will also increase (Rigler 1964; Peters 1975, 1979; Levine and Schindler 1980; White et al. 1982).

To determine if zooplankton affect the P status of the phytoplankton, we tested the hypothesis that reducing zooplankton biomass would result in an increase in k and that the magnitude of this increase should be greater than the observed increase in total phytoplankton biomass. This type of response would yield communities with a lower turnover time of P per unit algal biomass which are comparatively more P-deficient.

This prediction assumes that k increases linearly with the natural logarithm (ln) of total algal biomass. This assumption is introduced in calculating k from compartmental exchange models (Riggs 1963; Rigler 1973; Norman and Sager 1978). It has empirical support (Faust and Correll 1976; White et al. 1982), and this assumption is common to all estimates of state variables corrected for biomass, e.g. alkaline phosphatase activity (Sproule and Kalff 1978; Smith and Kalff 1981) and biomass-specific production rates and nutrient uptake (Richey 1979; Lehman and Sandgren 1982). The rate constant (k) expressed per unit biomass has been proposed as an indicator of P deficiency, and in Lake Memphremagog it correlates well with other indices of deficiency (e.g. alkaline phosphatase activity and boiling water extractable P) (Sproule and Kalff 1978; Smith and Kalff 1981).

Materials and Methods

To test our hypothesis, we conducted a series of seven field experiments during three consecutive seasons (1979, 1980, and 1981) in Lake Memphremagog, Quebec. The first three experiments were done in 1979 and a complete description of their protocol and design can be found in McCauley and Kalff (1987). Here, we present only their highlights. The experiments used 15 large-volume polyethylene enclosures, similar in design to those of McCauley and Briand (1979), in which biomass levels of crustacean zooplankton were systematically reduced by manipulation using a net (mesh size 75 μm). The enclosures were 1 m in diameter and 10 m deep, having an approximate volume of 7000 L.

A gradient of crustacean zooplankton biomass (from ambient to very low levels) was created initially in the 15 enclosures. Following the manipulation (for details of the size–structure and taxonomic composition of the zooplankton, see McCauley (1983)), initial estimates of phytoplankton biomass and species composition, zooplankton biomass, and the rate constant of orthophosphate uptake were made on samples from each enclosure. After 5–7 d, the enclosures were resampled and final values of these variables were obtained. Zooplankton were sampled by vertical net haul (mesh size 75 μm), and phytoplankton were sampled using a tube sampler. Phytoplankton were enumerated using the Utermöhl technique, and >1200 individuals (a minimum of 400 in each of the following groups: (1) algae >35 μm, (2) algae 10–35 μm, and (3) algae <10 μm) were counted in each sample. Crustacean zooplankton were counted (>400 individuals/sample) using a dissecting microscope and their biomass (dry weight) estimated using length–weight regressions (Bottrell et al. 1976). Average lengths were based on measuring more than 50 individuals of each species. Finally, the rate constant for orthophosphate uptake was determined using the radiobiological technique (see below).

TABLE 1. Initial values of chlorophyll a (μg/L) for control (enclosures 1, 2, and 3) and manipulated (enclosures 4, 5, and 6) treatments during the 1980 experiments. Mean estimates (X) and the 95% confidence limits (C.L.) are provided for replicate samples (n = 3).

	Exp. 1		Exp. 2		Exp. 3	
Enclosure	X	C.L.	X	C.L.	X	C.L.
1	2.33	0.25	3.15	0.08	4.14	0.03
2	3.53	0.48	2.64	0.44	4.53	0.10
3	2.88	0.30	2.83	0.35	4.24	0.10
4	2.44	0.15	2.66	0.05	5.13	0.17
5	2.44	0.21	3.26	0.29	3.90	0.14
6	2.29	0.25	3.46	0.38	4.26	0.04

By creating at one point in time a variety of systems with identical algal communities but differing levels of herbivores, we can monitor changes in the algal community and the rate of orthophosphate in response to this gradient of herbivore biomass. With this information, we can examine, using regression techniques, how the relation between algal biomass and orthophosphate uptake is affected by different levels of herbivores.

Three additional experiments were done in 1980 (16–24 June, 10–17 July, and 19–26 August) to test if the pattern observed in 1979 was consistent among years. These experiments (Table 1) used six enclosures (three control and three reduced zooplankton treatment). The protocol and sampling regime were identical to the previous experiments, with the exception that algal biomass was estimated as chlorophyll a (corrected for phaeophytin) using the acetone extraction procedure (Strickland and Parsons 1972; Smith 1981). McCauley and Kalff (1987) showed that there was no significant effect of the net manipulation on initial algal biomass in the three experiments done in 1979.

To estimate the rate constant for orthophosphate uptake (k), approximately 100 mL from freshly collected tube samples was used, with 5-mL subsamples taken following a geometric time series over a 3-h period (Peters 1975, 1979). These subsamples were filtered through a 0.45-μm membrane. The filters were then counted for $[^{32}P]PO_4$, using a Geiger-Mueller counter, and the percent of total radioactivity remaining in the filtrate was plotted against time. The initial slope converted to ln scale approximates the uptake rate constant (k, per minute) for orthophosphate. The reciprocal is the turnover time of orthophosphate in the sample: the time required for an amount of orthophosphate equivalent to that present to be taken up by the

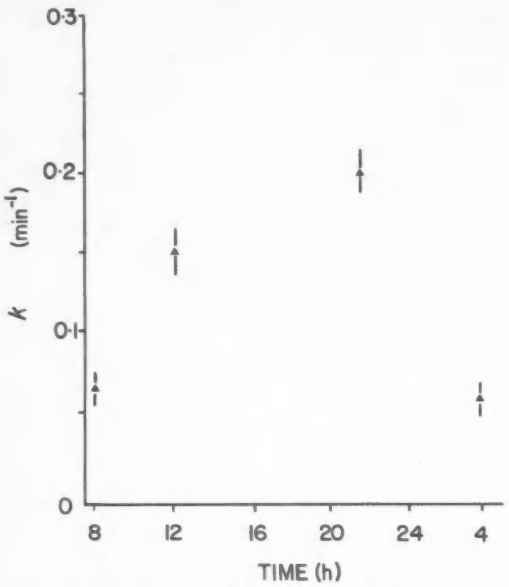

FIG. 1. Diurnal variation in the rate constant for orthophosphate uptake. Vertical error bars represent the 95% confidence limits of the mean estimated from three replicate tube samples.

particulate fraction. We found significant diurnal variation in k in lake samples (Fig. 1), and therefore, we adopted a constant sampling regime between 22:00 and 24:00. Sampling at night also prevented light shock to the phytoplankton.

The initial results (postmanipulation) for k in each enclosure experiment are listed in Table 2. For logistic reasons, only 10 enclosures were sampled for initial estimates of k. There was no significant initial effect (one-way ANOVA; $p > 0.05$) of the net manipulation on k in experiments.

To test the hypothesis that zooplankton significantly affect the P demand of phytoplankton, the relationship between k and various levels of zooplankton biomass was examined in the 1979 experiments. To compare increases in P demand with changes in algal biomass, the slopes of the relationships between turnover time versus zooplankton and total algal biomass versus zooplankton were tested for significant differences. Both the dependent variables were transformed to ln scale prior to regression analysis to remove scale effects in calculating the slopes.

In 1981, one experiment was done (23–27 June) to determine the size fractions that were most affected by changing zooplankton biomass. Size separations (>0.2 and >3.0 μm) were performed in the P uptake experiments. Three replicates of each sample were used. In Lake Memphremagog these separations allow adequate distinction of changes in the contribution of algae and bacteria to P uptake in Lake Memphremagog (cf. Currie and Kalff 1984, table 1). In addition, [^{14}C]glucose uptake was monitored on the respective size-classes to estimate bacterial activity (Berman and Stiller 1977).

Results

During the first experiment, reduction of zooplankton produced a significant increase in k (Fig. 2a). The line of best fit is

TABLE 2. Initial measurements of the rate constant for orthophosphate uptake (k, min^{-1}) in control and manipulated enclosures for each experiment performed in 1979 and 1980. Treatment groups represent the number of net hauls used in the manipulation of zooplankton biomass. F-values and significance are given for one-way analysis of variance procedures to test if treatment had an initial effect on k.

Treatment	Exp. 1	Exp. 2	Exp. 3
1979			
0 (control)	0.1415	0.0122	0.0406
	0.1284	0.0111	0.0509
		0.0132	0.0568
			0.0447
3	0.128	0.0123	0.0461
	0.136		0.0488
4	0.138	0.0116	0.0524
	0.143	0.0122	0.0375
	0.122	0.0111	0.0345
5	0.136	0.0111	0.0665
	0.142	0.0101	0.0332
	0.140	0.0132	
F	0.411 (ns)	0.518 (ns)	0.294 (ns)
1980			
0 (control)	0.0169	0.0864	0.0070
	0.0144	0.0839	0.0070
	0.0139	0.0998	0.0068
4	0.0170	0.0810	0.0066
	0.0153	0.0845	0.0068
	0.0153	0.0854	0.0077

$$(1)\quad k = 0.286 - 0.00301Z$$

where k is the rate constant (per minute) and Z is the zooplankton biomass (micrograms per litre).

The slope is significantly different from zero ($p > 0.0004$, SE = 0.0006) and the relationship is significant ($r = 0.869$). The observed variation in total phytoplankton (P) biomass (Fig. 3A) is also significant ($p > 0.0001$, $r = 0.917$, SE = 0.00283), but the slope of the relationship is not significantly (t-test; $p > 0.05$) from the slope of the relationship between turnover time (TT = $1/k$) and zooplankton biomass (Z):

$$(2)\quad \ln P = 8.277 - 0.01855Z$$

$$(3)\quad \ln \mathrm{TT} = 1.186 + 0.01633Z.$$

While the relationship between TT and Z is significant ($r = 0.872$, SE = 0.003), the change in turnover times upon reduction of zooplankton changed at the same rate as the increase in phytoplankton biomass.

A similar result was found for the second field experiment (Fig. 2B). Reducing zooplankton biomass again produced a significant increase in k ($p > 0.0005$, $r = 0.877$, SE = 0.00446):

$$(4)\quad k = 0.1055 - 0.02586 \ln Z.$$

The response of phytoplankton (Fig. 3B) was also significant ($p > 0.0001$, $r = 0.877$, SE = 0.06136) and the equation of best fit is:

$$(5)\quad \ln P = 6.252 - 0.3827 \ln Z.$$

However, the absolute value of the slope was not significantly different from the slope of the P turnover time – zooplankton biomass regression ($r = 0.878$, SE = 0.0789):

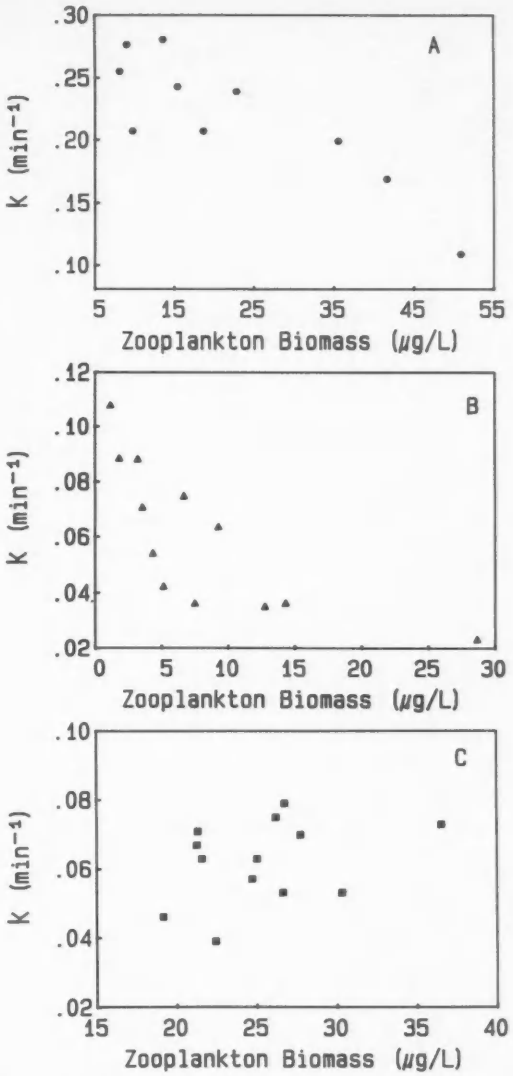

FIG. 2. Relationships between the rate constant for orthophosphorus uptake and zooplankton biomass for the three field experiments (A, B, and C) conducted during 1979.

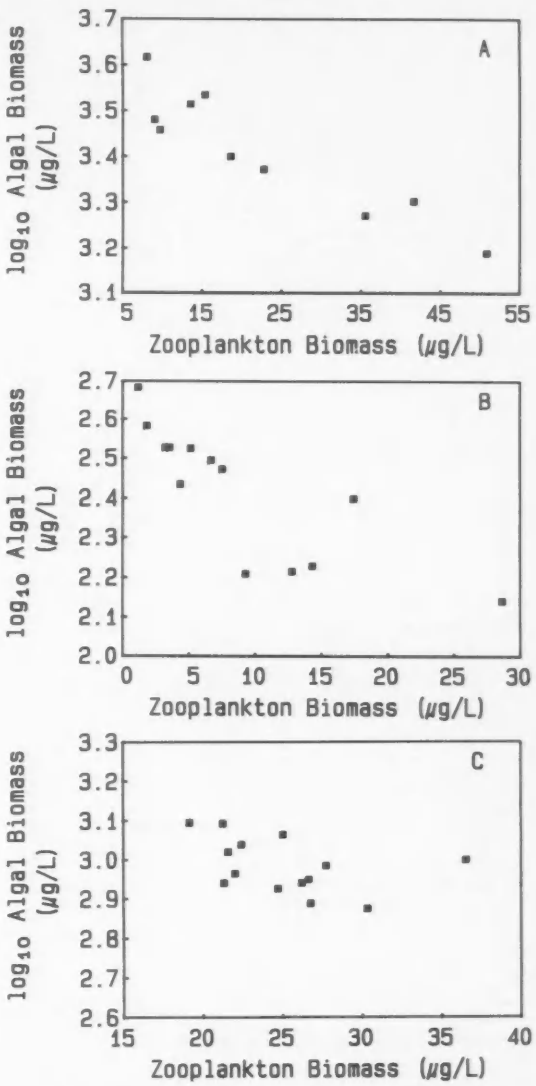

FIG. 3. Relationships between total phytoplankton biomass and zooplankton biomass measured after 5 d for the three field experiments (A, B, and C) conducted in 1979.

(6) $\ln TT = 2.101 + 0.460 \ln Z$.

As in the previous experiment, the magnitude of changes in turnover time and phytoplankton was not significantly different.

In the third field experiment, reducing zooplankton did not have a significant effect on k (Fig. 2C). There was no significant relationship between variation in k and zooplankton biomass ($p > 0.05$, $r = 0.346$). Although phytoplankton biomass appeared to increase under reduced zooplankton (Fig. 3C), there was no significant relationship ($p > 0.05$, $r = 0.423$) between variation in phytoplankton and zooplankton biomass. In this experiment, a significant reduction of zooplankton biomass did not result in either an increase in P demand or phytoplankton biomass.

During the 1980 and 1981 experiments, the rate constant was expressed per unit chlorophyll a (k'), which is a measure of P deficiency (Sproule and Kalff 1978). In the first experiment (Table 3), reducing zooplankton resulted in an increase in k' among enclosures. The changes in k' suggest that, unlike the previous experiments, reducing zooplankton biomass produces communities that are more P deficient at this time in the growing season. However, there was no significant effect ($p > 0.05$) on k' upon reduction of zooplankton in the second and third field experiments (Table 3). In June 1981, reducing zooplankton resulted in an increase in k', and size-fractionation studies show that this was the result of changes in the activity

TABLE 3. Final estimates for the rate constant of orthophosphate uptake (k') expressed per unit chlorophyll *a* in the 1980 and 1981 experiments. Zooplankton biomass (Z) is expressed in units of dry weight (μg/L). The rate of uptake per unit chlorophyll *a* of the particulate fraction >30 μm (k'_p) is presented for the 1981 experiment as well as the percentage of glucose uptake in this fraction. Values of k' and k should be multiplied by the magnification factor presented to get estimates in units/min.

	June		July		August	
Enclosure	Z	k' ($\times 10^{-2}$)	Z	k' ($\times 10^{-2}$)	Z	k' ($\times 10^{-2}$)
1980						
1	30.4	2.808	14.3	2.162	14.2	4.028
2	39.8	4.063	9.7	2.336	20.0	4.207
3	136.6	0.472	15.5	2.923	15.4	4.560
4	142.0	0.466	22.8	3.021	25.9	5.626
5	102.6	0.395	36.4	2.921	30.6	3.597
6			23.9	2.990	27.8	4.688

	Z (μg dry wt/L)	k ($\times 10^{-2}$)	k'_p ($\times 10^{-3}$)	% [^{14}C]glucose uptake
June 1981				
3	14.4	8.56	2.88	4.7
2	19.7	6.95	2.02	5.2
5	51.1	6.54	1.24	4.5
4	53.0	5.42	0.89	4.5
6	75.7	6.50	0.97	3.8

TABLE 4. Outcome of the field experiments as compared with the composition of the crustacean zooplankton during the experiment.

Experiment	Proportion of cladocerans	Change in cycling rate per unit algal biomass?
1. 1981	0.889	Yes
1. 1980	0.857	Yes
2. 1980	0.780	No
3. 1980	0.699	No
1. 1979	0.697	No
2. 1979	0.607	No
3. 1979	0.441	No

of the particulate fraction > 3.0 μm (Table 3). Over 95% of the bacterial activity, as measured by the uptake of [^{14}C]glucose, was recorded in the 0.2−3.0 μm fraction. There was no apparent increase of k' in the latter fraction and there was no difference in [^{14}C]glucose uptake between enclosures with ambient and much reduced levels of zooplankton biomass.

There appears to be a relationship between whether or not the cycling rate per unit algal biomass was affected and the taxonomic composition of the zooplankton. The cycling rate per unit algal biomass was only affected during experiments in which cladoceran zooplankton dominated the crustacean community, or conversely, when copepods were relatively less abundant (Table 4). However, it is important to note that the experiments in which a response was observed also had the highest biomass of zooplankton.

Discussion

The results show that crustacean zooplankton can significantly affect P demand as measured by the turnover time of orthophosphate. Two of three experiments in 1979 showed a reduction in turnover times among enclosures with reduction of herbivores. But, in both experiments, the increase in turnover time of orthophosphate was paralleled by an increase in phytoplankton biomass among enclosures. Thus, the cycling rate per unit phytoplankton biomass was not affected by reducing zooplankton biomass. If the turnover time per unit algal biomass is indicative of P deficiency (Sproule and Kalff 1978), then these experiments show that there was no change in the P deficiency of algal communities maintained for 5 d under conditions of reduced zooplankton biomass. Indeed, only two of the seven field experiments done provide evidence that reducing zooplankton leads to an increase in P deficiency.

Reducing natural levels of zooplankton decreases grazing rates and also the rate of nutrient regeneration in the community. If the supply rate of nutrients from zooplankton was important to a significant fraction of phytoplankton, then it is surprising that there could be a twofold increase in algal biomass among enclosures (experiencing reduced supply rates due to decreases in zooplankton biomass) without affecting the P deficiency of the plankton.

These observations indicate that (1) the magnitude of P regeneration by crustacean zooplankton was small relative to other processes (e.g. bacterial activity) or (2) P supplied by zooplankton is generally not important because the growth rates of a significant portion of phytoplankton are not limited by P in Lake Memphremagog or (3) there are strong and fast acting compensatory mechanisms either involving the algal community itself, or some other component of the community, that can buffer the perturbations in nutrient supply rates caused by manipulating zooplankton biomass. For example, reducing crustaceans could lead to the competitive release of rotifers or protozoans that could increase nutrient regeneration rates. While this did not occur for rotifers (E. McCauley, pers. obs.), we cannot eliminate the possibility for protozoans, as they were not adequately sampled. Since the rates of P regeneration,

measured on individual zooplankton species, are substantial (Peters and Rigler 1973; Devol 1979; Lehman 1980), the first possibility is most unlikely and we prefer the second explanation that zooplankton supplied P is of little consequence owing to conditions of P sufficiency. Additional observations support this explanation. During the 1979 experiments we observed growth of phytoplankton within the edible group in response to reduced zooplankton that is consistent with simple exponential growth models and inconsistent with models that assume growth limitation (McCauley and Kalff 1987). Also, Smith and Kalff (1981) concluded that growth rate limitation of phytoplankton is relatively mild, for most of the growing season, based on evidence from alkaline phosphatase activity. They reported that periods of greatest P limitation were restricted to early portions of the growing season, which corresponds well to the seasonal variation we observed.

These results question the importance of P regeneration rates by crustacean zooplankton during different times of the season in Lake Memphremagog. Elsewhere, Lehman (1978) observed that in Egg Lake, altering the abundance of crustacean zooplankton did not lead to changes in the dissolved P concentrations despite significant increases in algal biomass. He also observed that during this interval, certain algal species were reproducing at close to maximal rates, which indicates nutrient sufficiency rather than limitation. Conversely, Lehman (1978, 1980) found that regeneration of P by zooplankton was important in Lake Washington and in Sportsman Lake. It is clear from these studies that regeneration rates by zooplankton are of variable importance to phytoplankton dynamics.

The size-fractionation experiment showed that crustacean zooplankton did not have a significant effect on the activity of bacteria as estimated by glucose and P uptake. To our knowledge, this represents the only in situ test of the hypothesis that crustacean zooplankton are important in determining bacterial activity in lakes.

Our data suggest the following conceptual framework. At relatively high nutrient levels, zooplankton affect algal community structure by selectively grazing smaller phytoplankton (Porter 1973; McCauley and Kalff 1987) but do not materially affect nutrient supply rates to the phytoplankton. When algal growth rates are lowered with decreasing levels of total P (Smith and Kalff 1981), the effect of zooplankton on algal growth increases. However, with marked seasonal and temporal changes in algal growth (Smith and Kalff 1981), the role of zooplankton as providers of nutrients is, at any one time, probably best predicted from zooplankton biomass and a measure of the phytoplankton P status or community P turnover rate. The ability to predict the nature of the roles played by zooplankton is important in developing theories to explain both the short-term (McCauley and Kalff 1987) and long-term (Murdoch and McCauley 1985; McCauley and Murdoch 1987) dynamics of natural phytoplankton communities.

In conclusion, natural levels of zooplankton significantly reduce the P demand of phytoplankton communities as measured by the turnover time of orthophosphate. Communities maintained under reduced levels of zooplankton for 5 d sometimes showed signs of P deficiency; however, more often this effect was not observed. The equivocal nature of these results and similar ones from other lakes suggests that algal communities are frequently far from equilibrium with their resources.

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Enzyme-Linked Immunosorbent Assay for a Soluble Antigen of *Renibacterium salmoninarum*, the Causative Agent of Salmonid Bacterial Kidney Disease

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Pascho, R. J., and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. Can. J. Fish. Aquat. Sci. 44: 183–191.

A double-antibody enzyme-linked immunosorbent assay (ELISA) for detection of a soluble fraction of *Renibacterium salmoninarum* was developed from components extracted from the supernatant of an *R. salmoninarum* broth culture. The Costar® Serocluster™ EIA microplate gave the highest absorbance and signal-to-noise ratios among seven types tested. Including Tween 80 in the wash buffer resulted in higher absorbances than Tween 20 when antigen was present. Background absorbance did not increase when Tween 80 was added to the wash buffer, but did when Tween 80 replaced Tween 20 in antigen and conjugate diluents. Adsorption of coating antibody peaked within 4 h at 37°C and 16 h at 4°C. Antigen attachment to antibody-coated microplate wells depended more on incubation temperature than duration; we adopted a 3-h incubation at 25°C. Conjugate incubation for longer than 1 h at 37°C or 3 h at 25°C resulted in unacceptable background levels. No cross-reactions resulted from heat-extracted antigens of 10 other species of bacteria. The optimized ELISA is a 6-h test that enables detection of levels of soluble antigen as low as 2–20 ng.

On a mis au point un dosage immuno-enzymologique à anticorps double (ELISA) à partir de composantes extraites du surnageant d'une culture en bouillon de *Renibacterium salmoninarum*, dosage qui servira à la détection de la fraction soluble de la bactérie. Parmi les sept types de microplaques évalués, la microplaque Costar® Serocluster™ EIA a montré la plus grande capacité d'absorption et le rapport signal/bruit le plus élevé. L'apport de Tween 80 dans le tampon d'extraction a produit une plus grande capacité d'absorption que le Tween 20 quand des antigènes étaient présents. La capacité d'absorption ambiante n'a pas augmenté après l'ajout de Tween 80 au tampon d'extraction, mais elle l'a fait quand on a remplacé Tween 20 par Tween 80 dans les diluants d'antigènes et de conjugats. L'adsorption d'anticorps d'enrobement a culminé après 4 h à 37°C et après 16 h à 4°C. La fixation de l'antigène aux puits de microplaques recouverts d'anticorps dépend plus de la température d'incubation que de la durée; on a ainsi utilisé une incubation de 3 h à 25°C. L'incubation de conjugats pendant plus de 1 h à 37°C ou de 3 h à 25°C a produit des niveaux ambiants inacceptables. Aucune réaction croisée n'a eu lieu entre les antigènes obtenus par extraction thermique de 10 autres espèces de bactéries. La technique ELISA optimalisée peut s'effectuer en 6 h et permet la détection de faibles teneurs d'antigènes solubles (de 2 à 20 ng).

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Bacterial kidney disease (BKD), caused by the gram-positive bacillus *Renibacterium salmoninarum* (Sanders and Fryer 1980), is widespread geographically and is considered to be a major obstacle in the culture of Pacific salmon (Bell and Margolis 1976; Kimura 1978; Fryer and Sanders 1981). Mortality occurs in hatchery and wild juvenile salmon in both salt water and fresh water (Bell 1961; Ellis et al. 1978) and in prespawning adults (DeCew 1972). Successful enhancement of spring chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon populations may require the screening of every spawning adult to effect a reduction in vertical transmission of the bacterium. Such screening would require that the level of *R. salmoninarum* be measured in spawning populations ranging in size from 250 to 25 000 fish each.

Observation of clinical signs and Gram staining of tissue smears suffice to diagnose acute BKD but not subclinical infections. The bacterium is slow growing and fastidious in its growth requirements on artificial media, apparently requiring cysteine (Ordal and Earp 1956) and serum (Evelyn 1977). The KDM 2 medium of Evelyn (1977) has been the most commonly used culture medium, but a semidefined medium was also described by Embley et al. (1982). Culture has not been widely used for detection of BKD infection because several weeks of incubation are required for growth to occur when few bacteria are present, and during this time the culture is often contaminated by more rapidly growing microorganisms. A selective medium containing four antimicrobial compounds has been reported that will support the growth of *R. salmoninarum* while inhibiting that of most other microbial contaminants (Austin et al. 1983).

The direct and indirect fluorescent antibody tests are presently the most commonly used assays for detection of *R.*

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salmoninarum (Bullock and Stuckey 1975; Bullock et al. 1980). Although the indirect test is very rapid and is more sensitive than the Gram stain method, at least 10^4 bacteria must be present on the smear to assure a positive test (Bullock et al. 1980).

Chen et al. (1974) developed an immunodiffusion technique for diagnosis of overt cases of BKD in 24 h. This test enables detection of a heat-stable antigen (Kimura et al. 1978) that occurs in 10 strains of *R. salmoninarum* from North America (Bullock et al. 1974). Getchell et al. (1985) reported that a similar, heat-stable antigen was a common component of seven isolates of *R. salmoninarum*. A heat-stable antigen is also the basis for detection of low levels of BKD by coagglutination (Kimura 1978; Kimura and Yoshimizu 1981) and counterimmunoelectrophoresis (Cipriano et al. 1985), both of which are more sensitive than either Gram stain or immunodiffusion.

Enzyme-linked immunosorbent assays (ELISA) offer a combination of speed and sensitivity for detection of antigens (Ristaino et al. 1983; Matsumura et al. 1984; DeBoer and Osterhaus 1985) and can be partly automated for large-scale testing (Ruitenberg et al. 1977). Detection of a specific soluble fraction of *R. salmoninarum* offers a substitute for determining levels of the actual organism. An automated immunoassay for detection of that antigen would satisfy the requirements for 1-d processing of large numbers of samples on a quantitative basis. In this report we describe the development and testing of an ELISA for detecting low levels of a soluble fraction of *R. salmoninarum*. Because the ELISA technique is sensitive to procedural variations, we examined test variables and stabilized the assay to enable us to reliably detect the lowest possible level of BKD antigen.

Materials and Methods

Preparation of Soluble BKD Fraction and Antibody

Preparation of the extracellular fraction (designated 40SM antigen) was modified from a procedure previously developed by E. T. Manfredi (Biotechnics Inc., Redmond, WA 98052, USA, unpubl. data). Briefly, a pure culture of *R. salmoninarum*, isolate CC-80-369, from coho salmon was used to inoculate KDM 2 broth (Evelyn 1977) modified by the substitution of 10% normal rabbit serum for fetal bovine serum. Cultures were stirred from 10 to 14 d at 15°C and then centrifuged at 10 500 × *g* for 10 min at 4°C. The supernatant was lyophilized or stored at 4°C after the addition of 0.01% (w/v) merthiolate. For purification of the antigen, the lyophilized growth medium was rehydrated to a 4% (w/v) solution with distilled water. An equal volume of saturated ammonium sulfate (SAS) was slowly added and the mixture was stirred for 16 h at 4°C and then centrifuged at 12 000 × *g* for 10 min. The pellet was resuspended to the original volume with 40% SAS, stirred for 3 h at 4°C, and again centrifuged as above. The pellet was dissolved in distilled water, dialyzed against running tap water for 16 h at 4°C, and lyophilized after the addition of 0.01% (w/v) merthiolate.

New Zealand white rabbits were immunized with 1.0 mL of 40SM antigen (1.0 mg/mL), emulsified with an equal amount of Freund's complete adjuvant (Difco), in a series of intradermal injections in the scapular region. Intramuscular injections of 1 mL of 40SM antigen (1.0 mg) in Freund's incomplete adjuvant were given 14 and 28 d after the first injections. Blood was taken 14 d after the last injection and at weekly intervals thereafter for at least 3 wk.

We purified antibody by column chromatography using DE-AE AFFI-Gel® Blue (Bio-Rad Laboratories). Rabbit serum was dialyzed against 0.028 *M* NaCl with 0.02 *M* Tris−HCl (pH 8.0) overnight at 4°C and filtered through a 0.2-μm membrane filter (Gelman). Serum was applied to the column, the unbound protein peak was identified by measuring absorbance at 280 nm, and a volume equal to eight times the starting volume of serum was collected. The sample was reduced to half the initial serum volume by ultrafiltration (Amicon), sterilized by membrane filtration, and stored at −70°C with added merthiolate. The method of Nakane and Kawaoi (1974) as modified by Saunders (1979) was used to conjugate 40SM antibody to horseradish peroxidase (Sigma®).

We evaluated the relative strength of each purified antibody preparation by counterimmunoelectrophoresis (CIE) using various dilutions of 40SM antigen (1.0, 0.5, 0.1, 0.05, 0.025, 0.012, and 0.006 mg/mL). A 1.5-mm layer of 0.75% agarose (high electroendosmotic flow; Sigma®) in veronal buffer (pH 8.6, 0.1 ionic strength) containing 0.5% (v/v) Triton *X*-100 (Sigma®) was poured on glass slides precoated with 0.5% agarose. Antigen and antibody wells (2 mm) were punched in the gel 3 mm apart (edge-to-edge) and electrophoresis was done for 30 min at 6−8 V/cm. Slides were examined for bands before and after staining with Coomassie brilliant blue.

Optimization of the ELISA for 40SM Antigen

The ELISA developed to detect the 40SM antigen used the double-antibody sandwich design. Two equipment systems were used: a cuvette analyzer (Gilford® PR-50; used for evaluating the effect of detergent in the wash buffer and for comparing Tween 20 with Tween 80 in antigen and conjugate diluents only) and a 96-well microplate system (Titertek®; Flow Laboratories). We prepared carbonate-bicarbonate buffer, pH 9.6 (coating buffer); phosphate-buffered saline (PBS) with 0.05% Tween 20, pH 7.4 (PBS-T20); and phosphate−citrate buffer, pH 5.0, as described by Voller et al. (1979). All 40SM antigen dilutions were done in PBS-T20 unless otherwise indicated. The reagent volumes for the cuvette system were 300 μL for the coating antibody and 250 μL for the antigen and conjugate; 200 μL of the principal reagents were used in the 96-well microplate system. The system used for each experiment is identified by the terms cuvette or microplate well.

Cuvette or microplate well surfaces were coated by filling them with 40SM antibody in coating buffer, incubating them for 16 h at 4°C, and then rinsing them five times with wash buffer and once with distilled water. In initial experiments, done with the cuvette system, cuvettes were coated with antibody at 5 μg/mL and the conjugate dilution was standardized at 1:1200. Antigen was diluted in PBS-T20, added to the appropriate wells, and incubated at room temperature for 2 h. Control wells (blanks) were coated with 40SM antibody but received PBS-T20 without antigen. The wells were washed as described above, conjugate diluted in PBS-T20 was added to each well, and the plates were incubated at room temperature for 2 h. Unbound conjugate was removed by washing and the substrate−chromogen solution (50 mL of phosphate−citrate buffer at pH 5, 40 μL of 30% H_2O_2, 8 mg of *O*-phenylenediamine) was added (200 μL). Color development was stopped after 40 min at 37°C by the addition of 50 μL of 2 *M*

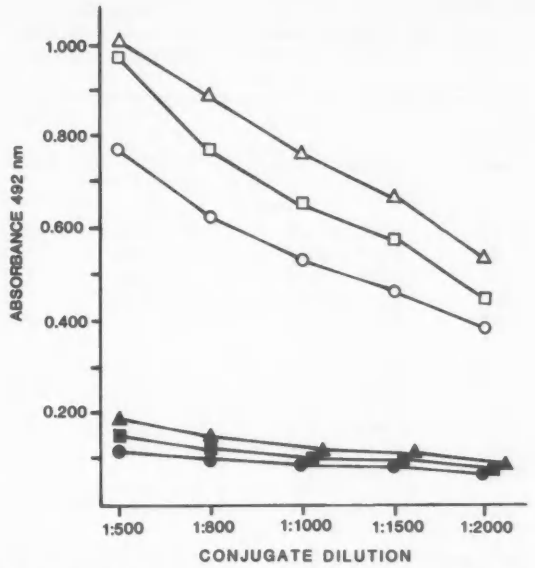

FIG. 1. Determination of coating antibody and conjugate dilutions for detection of 40SM antigen by ELISA. Antigen held constant at 200 ng/well. Coating antibody concentrations: ○, 2 μg/mL; □, 5 μg/mL; △, 10 μg/mL. Controls (solid symbols) corresponding to each level of antibody received PBS−T20 in place of antigen. All points are the mean absorbances of four replicates; SEM for all points was between ±0.0005 and ±0.0100.

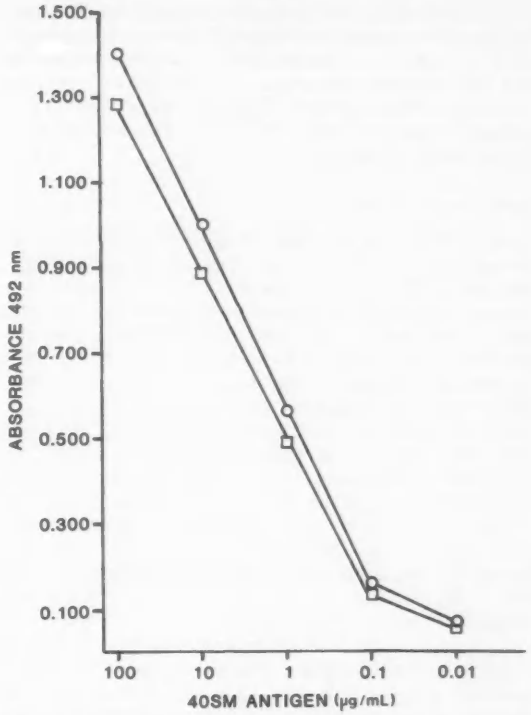

FIG. 2. Comparison of detection of 40SM antigen with conjugate diluted to two levels: ○, 1:1500; □, 1:2000. Coating antibody constant at 5 μg/mL. All points are mean absorbances of four replicates; SEM for all points was between ±0.0015 and ±0.0240.

H_2SO_4. Absorbances were read at 490 nm (cuvette system) or 492 nm (microplate system). The signal-to-noise (S/N) ratio (mean absorbance in wells containing antigen/mean absorbance of the blank) was calculated; a S/N ratio of approximately 2.0 was considered the minimum positive reaction.

We compared PBS wash buffers containing either 0.05% Tween 20 or 0.05% Tween 80 for their effect on the sensitivity of ELISA in detecting decreasing concentrations of 40SM antigen. Each wash buffer was substituted in the wash steps following incubations of the coating antibody, antigen, and conjugate. Purified 40SM antigen was diluted to 10, 1, 0.1, 0.01, and 0.001 μg/mL in PBS containing 0.05% Tween 20.

The diluents originally used for antigen and conjugate contained 0.05% Tween 20. Substitutions of Tween 80 for Tween 20 were made in either or both antigen and conjugate diluents in a comparison experiment. The 40SM antigen was added to cuvettes at 10, 1.0, and 0.1 μg/mL.

Seven varieties of microplates from four manufacturers were compared in the detection of 40SM antigen by ELISA. Coating antibody concentration and conjugate dilution were done as described for the cuvette system. The 40SM antigen was added to antibody-sensitized microplate wells at 100, 10, 1.0, and 0.1 μg/mL and PBS-T20 was added to blank wells. After completing the standard ELISA, we compared relative mean absorbances at each antigen concentration.

The concentrations of coating antibody and conjugate giving the highest S/N ratio were determined in one experiment. Anti-40SM antibody was diluted in coating buffer to 2, 5, and 10 μg/mL and microplate wells were coated with antibody overnight at 4°C. We washed the microplates and added antigen at 1 μg/mL (200 ng/well) to the test wells; control wells received buffer only. After the materials had been held for 2 h at room temperature, we washed the wells and added conjugate diluted 1:500, 1:800, 1:1000, 1:1500, and 1:2000. Incubation, washing, and color development were done as described earlier.

A second experiment was necessary to define the effects of high dilution of the conjugate on detection of very low antigen levels. With coating antibody concentration held constant at 5 μg/mL, we diluted the conjugate to 1:1500 and 1:2000 and used it to detect 40SM antigen diluted to 100, 10, 1, 0.1, and 0.01 μg/mL.

We next measured the effect of temperature and time on the adsorption of coating antibody. Microplate wells were sensitized with 1 ng antibody/well in coating buffer at 4 and 37°C. Incubation times ranged from 0.5 to 24 h. Antibody was added to the wells at intervals so that different incubation times ended simultaneously. The standard ELISA procedure was followed for the detection of 1 μg/mL (200 ng/well) of 40SM antigen.

In order to establish optimum time and temperature for antigen incubation, microplate wells were sensitized with 5 μg coating antibody/mL for 16 h at 4°C. Purified 40SM antigen at 100, 10, 1.0, 0.1, and 0.01 μg/mL in PBS-T20 was applied to sensitized plates and incubated at 4, 25, and 37°C. At intervals of 0.5, 1, 2, 3, and 4 h, we washed one plate (containing all antigen concentrations) and held it at 4°C until all incubation times had been completed; the ELISA was then completed. One plate, incubated for 24 h, was completed the following day.

Optimum conjugate incubation time was established by incubating antibody-coated wells with 40SM antigen at 1 μg/mL in PBS-T20. After a 2-h incubation, the plates were washed and each well received conjugate at a 1:2000 dilution. One plate was used per time interval at each temperature. The plates were washed at either 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, or 6.0 h and the ELISA assays completed.

Specificity of ELISA

All bacteria tested were cultured on KDM2 agar. Organisms normally associated with fish were incubated at 18°C and those associated with humans were incubated at 37°C. Bacteria were scraped from the agar surface and suspended in PBS-T20 to an optical density of 0.100 at 545 nm. The 40SM antigen contains heat-stable components of *R. salmoninarum* that have been shown to be serologically related to those found in at least the serum, feces, and kidneys of infected chinook salmon (E. T. Manfredi, Biotechnics Inc., Redmond, WA 98052, USA, unpubl. data). Therefore, we heated the suspensions at 100°C for 15 min and centrifuged at 11 800 × *g* for 5 min. We tested the supernatants using the final ELISA technique as follows.

(1) Coat wells of a Costar® Serocluster™ EIA microplate with purified specific rabbit antibody in carbonate–bicarbonate coating buffer at pH 9.6. (The volume of reagents, unless otherwise stated, is 200 μL.) Incubate 16 h at 4°C in humid chamber.

(2) Remove unbound antibody with five PBS-T80 washes with 30-s soaks each time the wells are refilled, followed by a final rinse with distilled water (again with a 30-s soak). This procedure is used in all wash steps mentioned below.

(3) Apply antigen diluted in PBS-T20 to test wells and only PBS-T20 to the blank wells. Incubate 3 h at 25°C and wash.

(4) Add diluted conjugate to all wells and incubate at 25°C for 2 h; wash.

(5) Add substrate and incubate at 37°C for 40 min. Stop reaction with 50 μL of 2 *M* H_2SO_4. Read absorbance at 490 nm.

Results

Preparation of Soluble BKD Fraction and Antibody

The 40SM antigen was present in high concentration in the pellet resulting from the 40% SAS precipitation. When equivalent amounts of lyophylized material from the pellet and the supernatant were used for immunization of rabbits, the latter resulted in a poor yield of antibody (data not shown). Lyophilization of the 40SM antigen did not affect its antigenicity.

Each lot of serum from rabbits immunized with the 40SM antigen was screened by CIE against dilutions of 40SM antigen to determine its potency. We made a total of five antibody purification runs using affinity column chromatography. The purified rabbit immunoglobulin from each column run was tested against increasing dilutions of 40SM antigen. Immunoglobulin from three of the five column runs reacted with the 40SM antigen at the lowest level tested (6 μg/mL); these three runs were pooled for use as coating antibody and for conjugation with enzyme.

Optimization of the ELISA for 40SM Antigen

Coating antibody concentration and conjugate dilution were determined together. Absorbances decreased with increasing dilution of the coating antibody, an effect also seen to a lesser degree in the controls (Fig. 1). Increasing the dilution of the conjugate also decreased the absorbance of both antigen and control wells. Selection of the best combination of coating antibody concentration and dilution of conjugate was based on keeping the absorbance of wells containing antigen at the 1 μg/mL level near 0.500 and keeping the absorbance of the controls below 0.100. At these values, antigen present at very low levels was more easily distinguished from background absorbance. Consequently, we selected a coating antibody concentration of 5 μg/mL. Because the two highest dilutions of conjugate (1:1500 and 1:2000) both gave acceptable results, we carried out a second experiment to compare the two dilutions in the detection of very low levels of 40SM antigen. The 40SM antigen was detected at all levels except 0.01 μg/mL with both conjugate dilutions (Fig. 2). In this experiment the limit of antigen detection that gave absorbances above 0.100 was 0.1 μg/mL (20 ng/well). The more economical 1:2000 dilution of conjugate was chosen for all further work because it gave nearly the same absorbance and *S/N* values as the 1:1500 dilution.

TABLE 1. Effect of the inclusion of Tween 20 or Tween 80 detergents in the wash buffer (PBS) on mean absorbance levels and signal-to-noise (*S/N*) ratios with various amounts of 40SM antigen (absorbance 490 nm).

40SM antigen (μg/mL)	Blanks[a]	40SM[a]	*S/N*
	Tween 80		
10	0.061 (0.004)	1.016 (0.015)	16.66
1	0.055 (0.002)	0.521 (0.005)	9.47
0.1	0.068 (0.003)	0.172 (0.006)	2.53
0.01	0.071 (0.004)	0.092 (0.003)	1.30
0.001	0.063 (0.003)	0.073 (0.002)	1.16
	Tween 20		
10	0.061 (0.003)	0.847 (0.007)	13.89
1	0.075 (0.004)	0.454 (0.007)	6.05
0.1	0.067 (0.005)	0.146 (0.002)	2.18
0.01	0.071 (0.006)	0.079 (0.003)	1.11
0.001	0.080 (0.003)	0.091 (0.002)	1.14

[a]Mean and SEM (in parentheses) for five replicates.

Inclusion of 0.05% Tween 80 in the wash buffer increased the absorbance values of the antigen-containing cuvettes, especially at higher antigen levels (Table 1). There was no difference in blank absorbance values when the wash buffer contained either Tween 20 or Tween 80; for both the limit of antigen detection in this experiment was 0.1 μg/mL (25 ng/well), which gave *S/N* ratios greater than 2.0. For subsequent experiments, the wash buffer was modified to include 0.05% Tween 80.

The background absorbances increased when Tween 80 was present in the antigen or conjugate diluents (Table 2). The use of Tween 20 in either or both diluents decreased absorbances in cuvettes containing antigen, but the *S/N* ratios remained high. The use of Tween 20 at 0.05% in both sample and conjugate diluents was adopted. The inclusion of high-molarity salt (0.5 *M* NaCl) in conjugate diluent as described by Saunders (1979) lowered the *S/N* ratios by increasing blank absorbances and decreasing absorbances in antigen-containing cuvettes (data not shown). High-molarity salt was not used further.

Performance of the microplate varieties tested for detection

TABLE 2. Effect of Tween 20 (20) or Tween 80 (80) in antigen and conjugate diluents on absorbances and S/N ratios with various levels of 40SM antigen. The diluent used was PBS with 0.05% (v/v) of either detergent added. Absorbance 490 nm.

40SM antigen (μg/mL)	Tween in diluent for: antigen	conjugate	Blanks[a]	40SM[a]	S/N
10	20	20	0.059 (0.002)	0.718 (0.043)	12.17
10	80	20	0.066 (0.001)	0.762 (0.058)	11.55
10	20	80	0.082 (0.004)	0.845 (0.028)	13.41
10	80	80	0.115 (0.003)	1.178 (0.008)	10.24
1	20	20	0.048 (0.002)	0.317 (0.013)	6.60
1	80	20	0.059 (0.002)	0.423 (0.004)	7.17
1	20	80	0.080 (0.002)	0.428 (0.005)	5.35
1	80	80	0.100 (0.004)	0.620 (0.007)	6.20
0.1	20	20	0.043 (0.003)	0.098 (0.004)	2.28
0.1	80	20	0.064 (0.005)	0.144 (0.002)	2.25
0.1	20	80	0.068 (0.002)	0.138 (0.003)	2.03
0.1	80	80	0.131 (0.003)	0.268 (0.005)	2.05

[a]Mean and SEM (in parentheses) for five replicates.

TABLE 3. Comparison of seven microtiter plates in detecting the 40SM antigen of *R. salmoninarum* by ELISA. Coating antibody 5 μg/mL, with rabbit HRP-Ig (1:1200) using OPD:H_2O_2 incubation 40 min at 37°C. Absorbance 492 nm.

Microplate	Manufacturer and number[a]	40SM antigen (μg/mL)[b] 100	10	1	0.1	Blank
Serocluster™	Costar®	1.701 (0.010)	1.212 (0.004)	0.727 (0.008)	0.257 (0.013)	0.118 (0.000)
EIA	3590	14.41	10.27	6.16	2.18	
Immunoplate I	Nunc	1.636 (0.006)	1.149 (0.007)	0.688 (0.005)	0.255 (0.002)	0.130 (0.002)
	439454	12.58	8.84	5.29	1.96	
Immunoplate II	Nunc	1.652 (0.004)	1.174 (0.003)	0.694 (0.005)	0.256 (0.002)	0.128 (0.001)
	442404	12.91	9.17	5.45	2.00	
Immulon® 2	Dynatech	1.372 (0.012)	0.892 (0.010)	0.513 (0.008)	0.201 (0.002)	0.124 (0.001)
	011-010-3450	11.06	7.19	4.14	1.62	
Linbro™	Flow	1.270 (0.022)	0.827 (0.004)	0.524 (0.007)	0.215 (0.004)	0.132 (0.001)
Titertek® (PVP)[c]	76-374-05	9.62	6.27	3.97	1.63	
Immulon® 1	Dynatech®	0.649 (0.010)	0.355 (0.003)	0.198 (0.002)	0.114 (0.001)	0.092 (0.001)
	011-010-3350	7.05	3.86	2.15	1.24	
Linbro™ EIA	Flow	0.782 (0.006)	0.433 (0.004)	0.239 (0.004)	0.133 (0.002)	0.117 (0.000)
	76-381-04	6.68	3.70	2.04	1.14	

[a]Costar®, Cambridge, MA; Nunc, Vangard International, Neptune, NJ; Dynatech Laboratories, Inc., Alexandria, VA; Linbro™, Flow Laboratories, McLean, WV.
[b]Mean and SEM (in parentheses) for five replicates; signal-to-noise ratio (S/N) below.
[c]Polyvinylchloride; all other microplates were made of polystyrene.

of different levels of 40SM antigen varied greatly (Table 3). At each 40SM antigen concentration there was a difference of at least 100% in the absorbance values between the microplate with the best and that with the poorest performance. The Costar® Serocluster™ EIA plate gave higher absorbances and S/N ratios than any other microplate tested at all antigen concentrations and was therefore used in all later tests.

Coating antibody adsorbed to microplate wells more rapidly at 37°C than at 4°C (Fig. 3). Absorbances in antigen-containing wells peaked after 2–4 h of incubation at 37°C and decreased with longer incubation times at this temperature. A parallel decrease in blank well absorbances meant that S/N ratios remained fairly constant. Adsorption of antibody plateaued after 16 h at 4°C, with no decrease in absorbance seen in either antigen or blank wells with longer incubation periods.

We evaluated the effect of incubation of microplates at 4, 25, and 37°C up to 24 h after addition of 40SM antigen at 10, 1.0, 0.1, and 0.01 μg/mL. Absorbance levels for antigen concentrations above 0.01 μg/mL increased with incubation periods up to 24 h at all temperatures and at all antigen concentrations above 0.01 μg/mL (Table 4). Absorbances generally increased with increasing incubation temperatures at all but the lowest antigen concentration. At 0.1 μg/mL (25 ng/well of antigen, the lowest concentration of antigen detected in this experiment, minimum incubation times for acceptable S/N ratios could be clearly established; S/N ratios exceeded 2.0 at incubation periods of 4 h at 4°C, approximately 3 h at 25°C, and 2 h at 37°C. We selected a standard antigen incubation time of 3 h at 25°C.

We examined the effect of conjugate incubation temperature

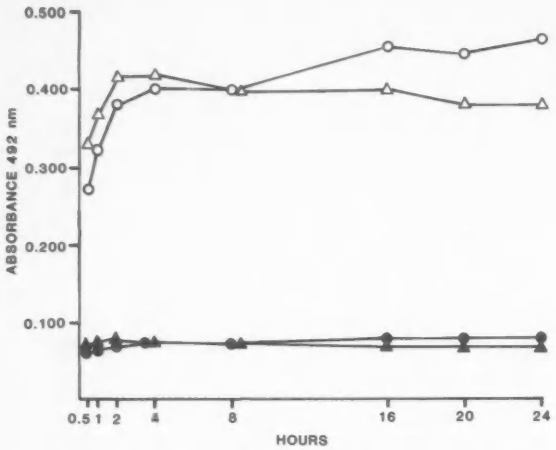

FIG. 3. Effect of incubation temperature on adsorption of coating antibody (5 μg/mL) to microplate wells. Temperatures: ○, 4°C; △, 37°C. Controls (solid symbols) corresponding to each level of conjugate received PBS−T20 in place of antigen. All points are mean absorbances of four replicates; SEM for all points was between ±0.0015 and ±0.0090.

and time on antigen detection using 1.0 μg/mL (200 ng/well) of 40SM antigen. The *S/N* ratios peaked after 1 h of incubation at 37°C and at 3 h at 25°C (Table 5). The drop in *S/N* ratio with increasing incubation times at both temperatures was the result of a substantial increase in the absorbance values in the blank wells beginning at 2 h at 37°C and 4 h at 25°C. Therefore, conjugate incubation times of 1 h at 37°C or 2 h at 25°C were chosen for all further work.

Only the heat-extracted antigens of the *R. salmoninarum* suspension resulted in a high absorbance level (Table 6). Supernatants of the other eight species produced no significant absorbance changes.

Discussion

We developed an ELISA to detect an antigen of *R. salmoninarum* and demonstrated that a rapid and sensitive test is possible. In the BKD−ELISA, success in detecting low levels of purified antigen was shown to be dependent on both the choice of microplate and correct adjustments to the basic double-antibody sandwich ELISA described by Voller et al. (1979).

The solid-phase support for ELISA is typically a polystyrene or polyvinyl chloride (PVC) 96-well microplate, although other supports are also used, including polystyrene beads (Miranda et al. 1977; Singh et al. 1984), dipsticks (Felgner 1978), polypropylene test tubes (Engvall and Perlmann 1971), and cuvettes, such as those used with the Gilford® components described earlier. The microplate format has gained the greatest popularity, and manufacturers have responsed by providing plates specifically designed and produced for the immunoassay. Our ELISA was first developed using the Gilford® cuvette system, but was adapted to the 96-well microplate format for economy of reagents and greater flexibility in the use of automated equipment that would permit the efficient testing of large numbers of samples. Variations in protein binding characteristics between microplates of different manufacturers

TABLE 4. Relationship of antigen incubation times and temperatures on the detection (expressed as signal-to-noise ratio) of five concentrations of 40SM antigen (nd = no data).

		40SM antigen (μg/mL)				
Temp. (°C)	Hours	100	10	1	0.1	0.01
4	0.5	13.36	8.69	3.80	1.35	1.04
	1.0	12.96	8.40	4.13	1.44	1.05
	2.0	15.32	9.75	5.25	1.65	1.05
	3.0	15.05	9.53	5.42	1.78	1.08
	4.0	15.94	10.07	6.07	2.06	1.10
	24.0	17.51	11.74	7.55	2.82	1.16
25	0.5	12.70	7.54	3.04	1.38	1.05
	1.0	15.86	10.21	5.27	1.66	1.07
	2.0	nd	nd	nd	nd	nd
	3.0	17.38	11.21	6.41	2.12	1.08
	4.0	17.17	11.18	6.61	2.28	1.12
	24.0	21.61	15.04	9.34	3.17	1.20
37	0.5	15.03	9.71	4.67	1.51	1.09
	1.0	17.03	11.83	6.01	1.79	1.06
	2.0	20.54	14.01	7.74	2.29	1.11
	3.0	20.94	14.44	8.46	2.60	1.14
	4.0	20.61	14.07	8.30	2.62	1.17
	24.0	23.04	17.76	10.59	3.04	1.15

have been reported (Kenny and Dunsmoor 1983; Shekarchi et al. 1984), emphasizing that plates from a number of sources must be screened for suitability in a given assay. Variations in absorbances between the seven plates we evaluated in the BKD−ELISA extend across all antigen concentrations tested and probably reflect differences in coating antibody binding among plates. These discrepancies directly affected the ability of the assay to detect the lowest antigen concentration, where the *S/N* ratios were only acceptable with the Nunc and Costar® plates. These levels were influenced by the high blank absorbances, a result of excessive coating antibody and conjugate concentrations that were established on the less sensitive Gilford® system. Future monitoring of fish populations for BKD will demand that the test used enable detection of very low levels of antigen, indicative of subclinical levels of infection. Because of its apparent suitability for this purpose, as indicated by the high *S/N* ratios with low levels of antigen, the Costar® 3590 Serocluster™ EIA Plate was selected for further use in the BKD−ELISA.

The final coating antibody and conjugate dilutions represented a balance between absorbance levels in the presence and in the absence of antigen. Increasing the conjugate dilution resulted in a decrease in the absorbance values of the blank wells. This decrease suggested that there was nonspecific binding of the conjugate to both the attached coating antibody and unoccupied cuvette or well surfaces. Conjugate affinity for proteins appeared to be greater than for plastic surfaces because our previous attempts at blocking unoccupied binding sites with bovine serum albumin increased the background beyond acceptable levels (data not shown). Kenny and Dunsmoor (1983) showed that the degree of albumin adsorption to well surfaces is related to the type of microplate used and that the amount of albumin that is bound directly influences nonspecific adsorption of conjugate. These authors demonstrated that the Costar® Serocluster™ EIA plate bound bovine serum albumin very effectively and consequently increased background absorbances. This increase probably accounted for our blocking

TABLE 5. Effect of conjugate incubation times and temperatures on detection of 1.0 μg/mL (200 ng/well) of 40SM antigen (absorbance 490 nm).

Time (h)	25°C			37°C		
	Blanks[a]	40SM[a]	S/N	Blanks[a]	40SM[a]	S/N
0.25	0.056 (0.003)	0.142 (0.003)	2.54	0.051 (0.001)	0.134 (0.001)	2.63
0.5	0.055 (0.002)	0.185 (0.001)	3.36	0.057 (0.001)	0.215 (0.002)	3.77
1.0	0.064 (0.001)	0.317 (0.003)	4.95	0.071 (0.001)	0.309 (0.004)	4.35
2.0	0.082 (0.001)	0.453 (0.003)	5.52	0.122 (0.001)	0.472 (0.004)	3.87
3.0	0.095 (0.003)	0.558 (0.009)	5.87	0.143 (0.001)	0.530 (0.007)	3.71
4.0	0.113 (0.001)	0.619 (0.004)	5.48	0.150 (0.002)	0.578 (0.004)	3.85
6.0	0.150 (0.002)	0.701 (0.000)	4.67	0.205 (0.004)	0.643 (0.005)	3.14

[a]Mean and SEM (in parentheses) of four replicates.

TABLE 6. BKD-ELISA absorbance levels of heat-extracted antigens of 11 species of bacteria.

Bacterium	Origin[a]	ELISA absorbance[b]	S/N
Renibacterium salmoninarum	Coho salmon	0.983 (0.026)	14.04
Aeromonas salmonicida	Chinook salmon	0.083 (0.005)	1.19
Yersinia ruckeri	Coho salmon	0.073 (0.003)	1.04
Vibrio anguillarum	Coho salmon	0.078 (0.003)	1.11
Rothia dentocariosa	ATCC 14190	0.074 (0.003)	1.06
Bacillus sphaericus	ATCC 14577	0.069 (0.001)	0.99
Bacillus subtilis	UW	0.070 (0.001)	1.00
Corynebacterium pseudodiphtheriticum	UW	0.083 (0.003)	1.19
Streptococcus Group D	UW	0.074 (0.003)	1.06
Streptococcus sp.	UW	0.071 (0.001)	1.01
Pseudomonas fluorescens	UW	0.077 (0.003)	1.10
PBS-T20	—	0.070 (0.003)	

[a]Coho salmon = *Oncorhynchus kisutch*; chinook salmon = *Oncorhynchus tshawytscha*; UW = University of Washington, Department of Microbiology, teaching collection cultures.

[b]Mean and SEM (in parentheses) for four replicates.

results, since we were unable to demonstrate specific reactivity to albumin or other spent culture medium components in the coating antibody preparations.

Detergents are commonly added to wash buffers and sample diluents, primarily to reduce background absorbance levels (Voller et al. 1979), although they also prevent inactivation of the horseradish peroxidase conjugate by microplate surfaces (Berkowitz and Webert 1981). The effect of detergents on background absorbance levels is explained by their ability to reduce nonspecific adsorption of sample proteins (Voller et al. 1979; Saunders 1979) and conjugate (Kenny and Dunsmoor 1983). Tween 80 was a beneficial additive to the wash buffer in the BKD–ELISA, but not when added to either the antigen, the conjugate diluents, or both. Tween 20 was added to the conjugate diluents because its use kept the blank absorbance levels at a minimum.

Coating antibody is typically attached to the solid phase by an overnight incubation at 4°C in carbonate–bicarbonate coating buffer (pH 9.6). We tested antibody adsorption at 4°C to determine how long it took to reach maximum saturation of the well surfaces and at 37°C to see if saturation could be reached more rapidly by increasing the temperature of reaction. Any period over 16 h at 4°C provided maximum coating antibody adsorption. The fall in absorbance levels with long coating incubation periods at 37°C may be due to the evaporation of water from the wells, which condensed on the underside of the plastic film used to seal the plate. Such evaporation may have increased the antibody and salt concentrations to a point that resulted in desorption or aggregation of the coating antibody. Desorbed antibody or unattached aggregates would then be removed in the next wash step, lowering the actual amount of immunoglobulin attached to the well surfaces. For these reasons, we adopted a coating antibody period of 16 h of incubation at 4°C for convenience, although we have also used a 3.5-h incubation period at 37°C with excellent results.

Binding of purified antigen was more dependent on the length of the incubation period than on the temperature. The absorbance levels of the blanks did not change, regardless of time or temperature, suggesting that longer incubation periods result in more antigen being bound without increasing nonspecific adsorption in the blank wells. The BKD-ELISA could be completed in 1 d. A 2-d ELISA, resulting from the use of very long incubation periods, would be useful only if a significant increase in sensitivity were gained. Extending the antigen incubation period to 24 h resulted in no significant increase in absorbance levels at the lowest antigen concentration (0.01 μg/mL) compared with incubation periods of 0.5–4 h. Sensitivity at this level of antigen could only be improved by greater antibody affinity, not by longer antigen incubations. Because incubation time and absorbance levels at the next

highest antigen concentration (0.1 μg/mL) were correlated, the apparent level of sensitivity of the BKD-ELISA was between 2 and 20 ng. In this range of antigen concentrations, the coating antibody became limiting because its affinity established how much antigen was retained for presentation to the conjugate. Nonspecific adsorption of conjugate became a significant factor when we compared conjugate incubation periods at 25 and 37°C. The degree of nonspecific binding during the adsorption period increased with time, as evidenced by higher blank absorbance levels and lower S/N ratios. The optimum conjugate incubation period was judged to be that which gave the highest S/N ratios and still enabled the test to be performed in 1 d. For our final BKD-ELISA procedure, we held the coating antibody and conjugate concentrations at the levels established for the original titration (5 μg/mL and 1:2000, respectively), using incubation times and temperatures at each step to maximize the S/N ratios at low antigen concentrations.

Reactivity of heat-extracted antigens from all 11 species of bacteria tested, except those from *R. salmoninarum*, was negligible in the final BKD–ELISA. There were small increases in absorbance levels from the non-*R. salmoninarum* species in comparison with the PBS–T20. These increases were attributed to a slight increase in nonspecific conjugate binding to extracted material that has adsorbed to the microplate surface. Dixon (1985) reported the development of an ELISA for *R. salmoninarum*, but its specificity was compromised because of cross-reacting antigens from *Rothia dentocariosa* and *Bacillus sphaericus*. Results indicate that our BKD-ELISA does not react with heat-extracted antigens from either bacterium, nor does it react with those of three common fish pathogens, *Aeromonas salmonicida, Yersinia ruckeri,* and *Vibrio anguillarum*. A *Corynebacterium* species was included because of the original classification of *R. salmoninarum* as a member of that genus (Ordal and Earp 1956); however, the antigen extracted from *C. pseudodiphtheriticum* did not react in the BKD-ELISA.

The BKD-ELISA has been used to demonstrate the presence of the 40SM antigen in the tissues and body fluids of salmon and trout infected with *R. salmoninarum*, and the results are presently being analyzed at this laboratory.

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Aggression and Variation in Growth of Chum Salmon (*Oncorhynchus keta*) Juveniles in Seawater: Effects of Limited Rations

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Chum salmon (*Oncorhynchus keta*) were grown in groups of 10 for 40 d on three limiting ration levels, and weight and fork length were measured at day 0 and day 40. Variation in growth of fish within a cohort was estimated from calculation of coefficients of variation for weight, fork length, condition factor, and growth rate. Average growth was significantly less at the lowest ration level than at the two higher levels, where average growth did not differ significantly. However, variation in growth (growth depensation) was significantly higher at the lowest and intermediate ration levels than at the highest ration level; we surmise that this higher variation is associated with increased competition and the disproportionate acquisition of food by larger fish. Aggressive behavior was most intense at the highest ration level. Chum salmon apparently show characteristics of a schooling fish while showing aggression, behaviors that are generally viewed as mutually exclusive.

Aux jours 0 et 40, on a déterminé le poids et la longueur à la fourche de saumons kétas (*Oncorhynchus keta*) élevés en groupes de 10 individus pendant 40 d et nourris de trois différentes rations. La variation de la croissance au sein d'une cohorte a été obtenue par calcul des coefficients de variation du poids, de la longueur à la fourche, du coefficient de condition et du taux de croissance. La croissance moyenne était nettement inférieure au plus faible niveau d'alimentation qu'aux deux autres niveaux plus élevés où la croissance moyenne n'était pas significativement différente. Par contre, la variation de la croissance était nettement plus marquée aux niveaux inférieur et intermédiaire d'alimentation; les auteurs formulent l'hypothèse que cette variation plus élevée est liée à une plus forte compétition et à la capture, par les plus gros poissons, d'une plus grande quantité de nourriture. Le comportement agressif était plus intense au niveau élevé d'alimentation. Le saumon kéta manifeste en apparence certaines caractéristiques d'un comportement de poissons en bancs tout en étant agressif, soit des comportements qui sont généralement considérés comme s'excluant réciproquement.

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Differences in the growth of fish of the same cohort may be caused by genetic differences and/or behavioral differences related to interference competition between individuals for food (for examples and discussion see Brown 1946; Magnuson 1962; Purdom 1974; Brett 1979; Jobling 1985; Koebele 1985). The intensity of interference competition depends largely on the behavioral repertoire of the species, with the keenest competition occurring in species that normally fight for food. The more limited or defensible the food supply, the more intense the competition resulting in an increase in growth variation over time (for examples see Brown 1946; Magnuson 1962; Nagoshi 1967; Purdom 1974; Yamagishi et al. 1974; Jobling 1985). Variation in growth or growth depensation is usually measured as an increase in the coefficient of variation of the size frequency distribution of a population over time (Brett 1979).

Based on observations in freshwater and seawater, the chum salmon (*Oncorhynchus keta*) has been characterized as a schooling species lacking intraspecific aggression (Hoar 1951, 1954; Shelbourn 1966). However, Mason (1974) and C. Groot (Pacific Biological Station, Nanaimo, B.C., pers. comm.) have incidentally observed aggression in the field and laboratory, respectively. Such interactions would seem inappropriate for a schooling species in which mutual attraction rather than aggression is expected to be the dominant behavior in social interaction. On the other hand, in nonschooling juveniles of coho (*O. kitsutch*) and chinook salmon (*O. tshawytscha*), aggression is linked to competition for food and space (Hoar 1951; Reimers 1968; Paszkowski and Olla 1985). If, in fact, behaviors that are generally viewed as mutually exclusive are present in chum, it would be informative to define when attraction and aggression are displayed, if aggression plays a role in competition for food, and how such interactions compare with those observed in other species.

The goal of this study was to describe social interactions and growth variation in chum salmon juveniles in seawater. Specifically we tested the hypotheses that limited ration levels affect (1) aggressive behavior and (2) interfish distance in chum and

that (3) growth variation is related to ration level. We also tested the effects of the presence of food and size of fish on intensity of aggressive behavior.

Materials and Methods

We conducted experiments on hatchery-reared underyearling chum salmon from Netarts Bay, Oregon, that had been transported to laboratory when they were 35 mm fork length (FL). The fish were reared in the laboratory in 20-L aquaria under seasonally adjusted photoperiod with filtered seawater at 10°C and $S = 32$ for 45 d until they reached a size of 60 mm FL. Fish were maintained on Oregon moist pellets (0.16 cm in diameter). Experiments were conducted in rectangular 340-L tanks (1.22 × 0.61 × 0.46 m) which had three opaque fiberglass sides and a transparent glass front. The tanks received a continuous flow (2–4 $L \cdot min^{-1}$) of filtered seawater from Yaquina Bay (11–30°C and $S = 30–32$). Fish were subjected to a seasonally adjusted photoperiod. Light was supplied to the tanks from cool-white fluorescent bulbs during the day and incandescent bulbs at night. Daytime light intensity (measured using a Li-cor quantum meter 188B and a 4π sensor) was 20.0 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ and 0.001 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ at night.

A second set of underyearling chum was used to compare behaviors of fish of different sizes. Chum were from Netarts Bay, Oregon, stock and were held in groups of 50–100 fish in 740-L circular flow-through tanks for 6 mo prior to experimentation. Filtered seawater was supplied as described above. Fish were fed Oregon moist pellets (0.32 cm in diameter) and reached a size of 135–150 mm FL. Experiments were conducted in rectangular 2004-L flow-through tanks (2.42 × 0.91 × 0.91 m) which had three opaque fiberglass sides and a transparent glass front. Light was supplied to the tanks as described above with an intensity of 8.0 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ during the day and 0.009 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ at night.

Experiments on the effects of ration level were conducted on the first set of chum in experimental tanks for a 40-d period. Ten fish were placed in each tank and two tanks were used for each of three treatments. Ration levels of Oregon moist pellets (0.16 cm in diameter) were 7.2% body weight (bw)$\cdot d^{-1}$ (A), 3.5% bw$\cdot d^{-1}$ (B), and 1.3% bw$\cdot d^{-1}$ (C). Chum were weighed to the nearest 0.01 g and measured to the nearest 0.1 cm at the beginning and end of the experimental period. Tanks were enclosed in a blind which permitted live observation of behavior for 10 min before and up to 90 min after introduction of food pellets. Pellets were introduced through a single feeding tube once each day. Video recordings (RCA model TC1005 ultracon camera taped at 1/72 normal speed) of the feeding trials were made simultaneously and later analyzed at normal speed for interfish distances.

Behaviors observed in groups of chum salmon consisted of fish typically maintaining themselves in a loose school and feeding with equal facility on pellets whether in the water column or on the bottom. Aggressive behavior consisted of chasing which occurred when a fish in the school swam after another fish. Both fish moved a short distance (three to five body lengths) from the school and then abruptly returned to the school ending the interaction. Nipping, other forms of aggressive display, or actual physical damage to fish during chasing were rarely observed. Fish were not observed to establish apparent territories at any time as compared with territories held by coho salmon in this same system (Paszkowski and Olla 1985).

An index of interfish distance (R) was calculated for two-dimensional measurements after Clark and Evans (1954) using the formula

$$(1) \quad R = \frac{\bar{r}}{E(r)}$$

where $\bar{r} = \Sigma r_i / N$, r_i = nearest neighbor distance between fish i and $i + 1$, N = number of fish in the tank, and

$$(2) \quad E(r) = \frac{1}{2p^{1/2}}$$

where p = density of population (number per area) using same units as for r_i. This index measures the types of distribution that can occur between objects, where $R < 1.0$ is an aggregated assemblage, $R > 1.0$ is a regular assemblage, and $R = 1.0$ is a random assemblage. For analysis, a single frame was selected at random for every 3 min of elapsed time. Nearest neighbor distances were measured between every fish and the index was calculated for that time frame. The inherent errors of measuring three-dimensional structures using two-dimensional data are discussed by Symons (1971a) and Dill et al. (1981).

Conditions factor (K) was calculated after Weatherly (1972) using the formula

$$(3) \quad K = \frac{100W}{L^3}$$

where W = weight (grams) and L = length (centimetres). Instantaneous growth rate (μ) was calculated using the formula

$$(4) \quad \mu = \frac{\ln (n_1/n_0)}{t_1 - t_0}$$

where n_0 and n_1 = initial and final weight or length, respectively, and t_0 and t_1 = initial and final time (days), respectively.

Experiments were designed to confirm the schooling and aggressive nature of larger chum (135–150 mm FL). Chum were placed in 2004-L experimental tanks in groups of four and six fish, and videotaped sequences 15 min in length were made at 1/72 normal speed at 1-h intervals around the clock. Eight replicate days were analyzed for each group size. Daily median values were used for statistical analysis. An observer also watched the fish for 15–20 min on a regular basis to confirm behavior recorded on videotapes. Chases and interfish distances were quantified. Nighttime sequences were not quantified because of technical difficulties.

We used Mann–Whitney U-tests, Wilcoxon matched pairs signed rank tests, Wilcoxon signed rank tests for independent samples, Kruskal–Wallis tests, and Friedman tests on observed values and medians to analyze patterns of weight, fork length, condition factor, growth rate, frequency of chases, and interfish distance (Daniel 1978; Sachs 1984). Patterns of coefficients of variation (CV = SD/$\bar{X}$) were analyzed for weight, fork length, and growth rate using Lohrding tests (Lohrding 1975). For all tests, the level of significance was set at $p < 0.05$.

Results

General Behavior

Aggressive behavior was always present in groups of fish of varying sizes (50–150 mm FL) maintained at varying densities (4–10 fish). No significant differences in frequency of chases were detected over the range of densities and sizes tested ($N = 8$, Friedman test, $p > 0.05$; Table 1).

TABLE 1. Daily median values for chases (per minute) and 95% confidence intervals for fish of different sizes (median size in group) held at different densities (4, 6, and 10 fish). Fish were observed for 15 min every daylight hour for 8 d.

	10 fish (55 mm)	10 fish (65 mm)	4 fish (140 mm)	6 fish (140 mm)
	3.6	3.2	2.5	2.4
	4.9	0.8	2.4	3.0
	0.1	4.3	2.4	4.9
	0.1	3.3	2.2	4.4
	1.4	3.4	2.1	3.2
	0.5	0.8	1.6	3.4
	1.9	5.0	2.3	3.8
	0.5	4.3	2.4	3.9
Median	1.0	3.4	2.4	3.6
95% CI	0.1–3.6	0.8–4.3	2.1–2.4	3.0–4.4

TABLE 2. Median numbers of fish chasing other individuals in 30 min for large (L), intermediate (I), and small (S) fish fed ration levels A (7.2% bw·d^{-1}), B (3.5% bw·d^{-1}), and C (1.3% bw·d^{-1}) on experiment days 0, 10, 18, 26, 33, and 40. Observations were made before, during, and after feeding. Fish sizes are relative within a replicate tank. No data available indicated by a dash; total number of observations = 15 for each ration level.

Experiment day	A			B			C		
	L	I	S	L	I	S	L	I	S
0	0	2	0	0	2	0	0	2	0
10	—	—	—	0	3	0	0	1	0
18	—	—	—	0	2	0	0	2	0
26	—	—	—	0	3	0	0	1	2
33	—	—	—	0	2	0	0	2	2
40	0	2	0	0	2	0	0	2	0

For the first group of fish (50–80 mm FL), larger fish did not chase other sized fish (Table 2). A significantly greater number of intermediate sized fish made chases than did smaller fish ($N = 14$, Wilcoxon test; Table 2). The terms large, intermediate, and small are relative sizes based on visual observation of fish in each replicate of each ration treatment. Because of difficulty recognizing individuals that were unmarked, we were unable to quantify our observations further.

Effects of Ration Level on Aggressive Behavior and Interfish Distance

Ration level appeared to affect the level of aggressive behavior in group 1 fish (50–80 mm FL; Table 3). A comparison of chases per minute between treatments A, B, and C on day 40 showed that significantly higher levels of aggression were associated with increased ration ($N = 3$, Friedman test; Table 3), with the presence or absence of food affecting the frequency of chases. For fish in treatment A on day 40, chases were significantly higher before food was present ($N = 4$, Friedman test; Table 3). Frequency of chases on days 10, 18, 26, 33, and 40 did not change for fish in treatment B until all food was ingested (see 21–30 min, Table 3) when chases increased significantly ($N = 5$, Friedman test; Table 3). Frequency of chases did not change significantly for fish in treatment C with the presence or absence of food ($N = 5$, Friedman test; Table 3).

Comparisons of interfish distance in this experiment were inconclusive; effects of ration level, presence of food, or day of experiments were not evident (Wilcoxon rank test, Friedman test, $p > 0.05$).

TABLE 3. Median numbers of chases (per minute) (1) and interfish distance (dimensionless) (2) for fish (50–80 mm FL) at ration levels A (7.2% bw·d^{-1}), B (3.5% bw·d^{-1}), and C (1.3% bw·d^{-1}) on experiment days 0, 10, 18, 26, 33, and 40. Food was introduced 11 min after observation began. Food was present for 3–7 min after introduction for all levels of feeding. No data available indicated by a dash.

Time (min)	Experiment day	A		B		C	
		1	2	1	2	1	2
Prior to food presentation (0–10)	0	0	0.58	0.2	0.44	0.1	0.79
	10	—	—	0.6	1.43	0.3	0.80
	18	—	—	0.3	0.72	0.5	0.84
	26	—	—	0.2	1.39	0.4	0.94
	33	—	—	0.2	0.81	0.3	0.89
	40	12.2	0.99	1.4	1.04	0.4	0.78
Food present (11–20)	0	3.5	0.56	1.0	0.34	1.2	0.37
	10	—	—	0.3	0.56	0.1	0.50
	18	—	—	1.3	0.50	0.2	0.75
	26	—	—	2.8	0.79	1.0	0.83
	33	—	—	0.2	0.81	0.3	0.89
	40	5.4	0.87	1.5	1.14	0.4	0.72
Food gone (21–30)	0	2.2	0.44	4.3	0.68	0.1	0.52
	10	—	—	0.8	0.63	0.2	0.66
	18	—	—	1.9	0.57	0.8	0.96
	26	—	—	3.0	1.08	1.5	1.17
	33	—	—	4.8	0.96	0.8	0.72
	40	7.4	1.19	2.8	1.24	0.5	0.64

Effects of Ration Level on Growth

After 40 d, fish fed 7.2% bw·d^{-1} (A) and 3.5% bw·d^{-1} (B) had increased in length and weight, but those fed at 1.3% bw·d^{-1} (C) had not ($N = 20$, U-test; Fig. 1). Fish from the two replicate groups of 10 fish were pooled for each treatment because there were no significant differences between replicates of a treatment. Fish from treatments A and B did not differ significantly in length or weight, but both were significantly larger than those from group C ($N = 20$, Kruskal–Wallis test).

While fish in treatments A and B did not differ in average growth, the coefficient of variation for weight and fork length was significantly greater in treatment B than in treatment A (Lohrding test; Table 4). Fish in treatment C also displayed significantly greater variation in weight than fish in treatment A (Lohrding test; Table 4).

Fish in treatment A displayed a significant increase in condition factor (CF) over 40 d. Fish in treatment B showed no change in CF and fish in treatment C showed a significant decrease in CF ($N = 20$, U-test; Table 4). At the beginning of the experiment, CF was equal between treatments ($N = 20$, Kruskal–Wallis test, $p > 0.05$). Condition factors were different between treatments at day 40, A > B > C ($N = 20$, Kruskal–Wallis test), and CF varied more in treatment C than treatments A and B (Lohrding test; Table 4).

Instantaneous growth rates were calculated using weight and FL on day 0 and day 40 for treatments A, B, and C (Table 4). This procedure assumed that the order of fish sizes within a treatment corresponded between days 0 and 40. No growth was

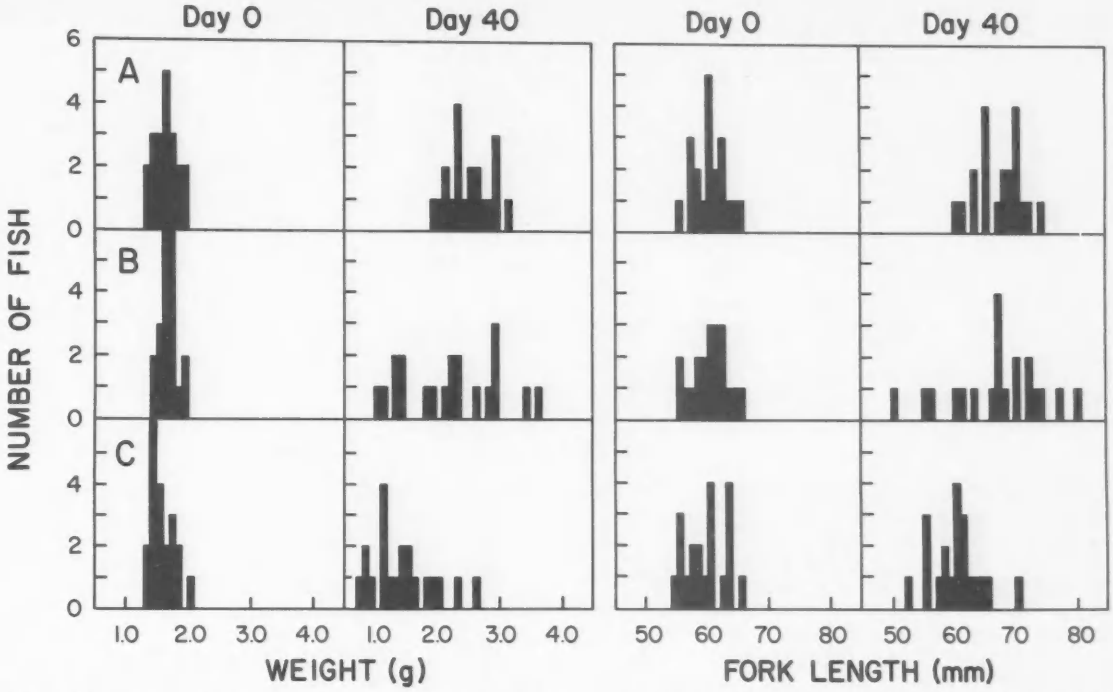

FIG. 1. Distribution of weight and fork length at day 0 and day 40 for chum salmon fed at ration levels A (7.2% bw·d^{-1}), B (3.5% bw·d^{-1}), and C (1.3% bw·d^{-1}).

evident for fish in treatment C. Fish in treatment A grew 1.1% bw·d^{-1} and 0.3% FL·d^{-1}, whereas fish in treatment B grew 0.7% bw·d^{-1} and 0.3% FL·d^{-1}. Median growth rates did not differ between treatments A and B, whereas in treatment C they were significantly less than for treatments A and B (N = 20, Kruskal–Wallis test; Table 4). The coefficient of variation for growth rates based on weight was significantly less for treatment A than for treatments B and C (Lohrding test; Table 4).

Discussion

In these experiments, chum at all three ration levels were considered to have a limited food supply based on the feeding and growth capabilities of the species at this life stage (LeBrasseur 1969; Volk et al. 1984). Because fish were always subjected to group interaction, our experiments did not allow us to determine the separate roles played by genetic and behavioral factors in differential growth. We assumed, however, that behavior significantly affected growth based on the increase in variance of growth over time (for examples and discussion see Magnuson 1962; Purdom 1974; Brett 1979; Jobling and Wandsvik 1983; Koebele 1985; Jobling 1985). We think that higher growth depensation at low and intermediate ration levels than at the high ration level would be due in large measure to increased competition and the disproportionate acquisition of food by larger fish.

The observed increase in growth depensation with decreased ration generally agreed with observations for medaka (*Oryzias latipes*) (Magnuson 1962), guppies (*Lebistes reticulatus*) (Nagoshi 1967), zebrafish (*Brachydanio rerio*) (Eaton and Farley 1974), and arctic charr (*Salvelinus alpinus*) (Jobling 1983, 1985). However, this relationship may not always be obtained because the degree of depensation can depend not only on the amount of food but on its defensibility and/or the degree that subordinate fish are suppressed in their foraging by dominants (for discussion see Magnuson 1962; Wilson 1975; Brett 1979; Koebele 1985). Thus, even in situations where the ration level may be considered to be in excess of the needs of the group, depensation may nevertheless be high, e.g. plaice (*Pleuronectes platessa*) (Purdom 1974), gobies (*Odontobutis obscurus*) (Yamagishi et al. 1974), and *Tilapia zilli* (Koebele 1985). An increase in growth depensation with time would therefore be indicative of competitive interactions but not necessarily of a decrease in food resources available to the whole population. Alternatively, for schooling fish such as sockeye salmon (*Oncorhynchus nerka*), decreasing ration did not produce an increase in growth depensation, as fish did not show aggressive behavior even under limited rations (Brett et al. 1969).

Since fairly high levels of agonistic behavior were observed for all three ration levels, it is reasonable to assume that this was the primary competitive mechanism. The larger fish, however, were not overtly aggressive and did not appear to interact actively with smaller fish, yet they obviously had the greatest access to food. In fact, most of the aggression that occurred was initiated by intermediate sized fish, suggesting that in chum salmon size alone may adequately communicate dominance and preclude overt or at least easily observable signs of aggression. These observations are in direct contrast with situations commonly reported for species that compete aggressively for limited food resources where socially dom-

TABLE 4. Initial (I) and day 40 (F) mean ($\bar{X}$) and coefficient of variation (SD/$\bar{X}$ = CV) for weight, fork length, condition factor, and growth rate of chum salmon (50–80 mm FL) grown 40 d at ration levels A (7.2% bw·d^{-1}), B (3.5% bw·d^{-1}), and C (1.3% bw·d^{-1}).

	A		B		C	
	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV
Weight						
I	1.64	0.12	1.68	0.09	1.53	0.16
F	2.51	0.14	2.38	0.35	1.45	0.36
Fork length						
I	60.1	0.04	59.9	0.05	59.2	0.06
F	67.3	0.06	66.9	0.11	59.8	0.07
Condition factor						
I	0.76	0.07	0.79	0.10	0.76	0.09
F	0.82	0.09	0.76	0.09	0.66	0.20
Growth rate						
Weight	0.011	0.09	0.007	1.00	−0.003	−2.00
Fork length	0.003	0.33	0.003	0.67	0.001	1.00

inant individuals attempt to control food sources (e.g. Magnuson 1962; Nagoshi 1967; Yamagishi et al. 1974; Fenderson et al. 1968; Keen 1982; Koebele 1985; Paszkowski and Olla 1985). Hoar (1951) recorded no aggressive interaction (as measured by nips) in chum fry held in freshwater and concluded that such behavior, when it does occur, may be characteristic of displacement behavior, i.e. behavior out of functional context. However, incidental observations in the field by Mason (1974) and in the laboratory by Groot (pers. comm.) indicated the presence of aggressive behavior in chum fry. In this present work, aggressive behavior was always present in fish at varying densities (4–10 fish) and sizes (50–150 mm). Comparison of chum behavior with behavior of coho smolts (Paszkowski and Olla 1985) showed that chasing was more frequency in chum, which formed schools, than in coho, which exhibited territorial spacing. In contrast, sockeye salmon and pink salmon (*Oncorhynchus gorbuscha*) appear to be schooling species showing little or no aggressive behavior, at least as juveniles in freshwater (Hoar 1954; Brett et al. 1969). Rather than aggression being characterized as displacement behavior in chum, we suggest that its presence or absence is more probably attributable to behavioral scaling, i.e. behavioral variation in magnitude or quality correlated in this case with environmental conditions (Wilson 1975).

Juvenile chum interacted aggressively in both the presence and absence of food but this behavior was not used in the establishment of a dominance hierarchy or in territorial defense, as was found for coho fry in freshwater (Hoar 1954; Chapman 1962), coho smolts in seawater (Mason 1966; Paszkowski and Olla 1985), and Atlantic salmon (*Salmo salar*) parr in freshwater (Symons 1968, 1971b). In fact, aside from the presence of interfish aggression in the absence of food, the overriding social behavior was mutual attraction, i.e. chum possessing the attributes of species which typically school (for example see Parr 1927; Breder 1959; Shaw 1970; Olla and Samet 1974). Hoar (1951) first characterized chum salmon as a schooling species based on laboratory observations of fry in freshwater. He described the fish as being mutually attracted, similarly oriented, uniformly spaced, and moving at uniform speed. Shelbourn (1966) also observed the predominance of schooling behavior but added that fry show a greater tendency to school in seawater than in freshwater. Except in cases where reproductive behavior was involved, e.g. Pacific bonito (*Sarda chiliensis*) (Magnuson and Prescott 1966), the presence of aggressive behavior in chum contradicts what has generally been observed in schooling species.

The response of chum interfish aggression to food stimulus was variable. At the highest ration, chasing was greatest before food introduction, while at the intermediate ration, chasing increased after all the food was eaten. In either case, chasing decreased while food was present. At the lowest ration, chasing did not change through the feeding cycle, probably resulting from the poor condition of the fish caused by food limitation. The presence of food produces varying effects on aggressive behavior in some other salmonids. In a review of foraging behavior in Atlantic salmon, coho salmon, and rainbow trout (*Salmo gairdneri*), Dill (1978, 1983) found that aggressive behavior may increase or decrease in the presence of food. These effects may be confounded by the effects of food on general activity, by threats of competition and predation, and by the condition of fish. Results of the present study suggest that a clear resolution of the effects of a food stimulus on aggressive behavior in territorial or schooling salmonids will depend on analysis of a suite of factors which affect the defensibility of food resources (for discussion see Magnuson 1962; Dill 1983; Koebele 1985).

Comparison of medians for weight, length, and growth rate between the two higher ration levels did not show any significant differences, although growth depensation, as measured by coefficients of variation for these variables, did differ. The effects of competition on growth are not necessarily demonstrated by consideration of median population growth, which assumes that competitive abilities of individuals are equal (Rubenstein 1981). Consideration of behavioral interactions and their effects on disproportionate food acquisition, assimilation, and growth are essential to understanding the role of individual variation in population dynamics; coefficients of variation of these variables are valuable indicators of competitive effects (Purdom 1974; Brett 1979; Rubenstein 1981; Jobling and Wandsvik 1983).

Our growth experiments were performed under conditions of constant fish density, habitat size and character, and food availability in a temporal and spatial sense. Variation and the interaction of these factors under natural conditions would produce a gradient of growth and behavioral responses that would reflect the depth of adaption in the species.

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Small-Scale Variability of New Production and Particulate Fluxes in the Ocean

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Vézina, A. F., and T. Platt. 1987. Small-scale variability of new production and particulate fluxes in the ocean. Can. J. Fish. Aquat. Sci. 44: 198–205.

Particulate carbon losses from the surface waters of the ocean can be estimated from short-term (≲1 d) determinations of the contribution of nitrate to total nitrogen uptake ($f(t)$), under the assumption that the nitrate flux represents the supply of N from external sources. We used numerical modeling of the euphotic food web to explore the relationship between short-term variability in $f(t)$ and the long-term export normalized to production, $\langle f \rangle$. We found that modeling sedimentation as a nonlinear process was necessary to simulate variability in $f(t)$. The resulting model generated nonlinear relationships of $f(t)$ to P_T and $f(t)$ to $[NO_3]$ that, to date, have only been empirically described. The shape of the f ratio curves depends on an exponent n that expresses a power law dependence of the sedimentation rate on the concentration of suspended material in the euphotic zone, for which there is increasing empirical support. Simulations of the model with a temporally variable NO_3 supply show that particulate losses can amount to 30–40% of production in seasonal oligotrophic systems. The model suggests that $\langle f \rangle$ in N-limited ocean provinces depends on the variance of the nutrient field and on the sensitivity of the loss rate to variations in the plankton biomass in the euphotic zone.

On peut mesurer les pertes de carbone particulaire dans les eaux océaniques superficielles en déterminant l'apport à court terme (≲1 d) de nitrate dans la captation totale d'azote ($f(t)$), d'après l'hypothèse que le flux de nitrate représente l'apport de N de sources externes. Les auteurs utilisent une modélisation numérique de la chaîne alimentaire de la zone euphotique pour étudier la relation entre la variabilité à court terme de $f(t)$ et l'exportation à long terme étalonnée en fonction de la production, soit $\langle f \rangle$. Ils notent qu'il est nécessaire de modéliser la sédimentation comme un processus non linéaire pour simuler la variabilité de $f(t)$. Le modèle obtenu a fourni des relations non linéaires entre $f(t)$ et P_t et entre $f(t)$ et $[NO_3]$ qui, jusqu'à maintenant, n'ont été décrites que de façon empirique. La forme des courbes du rapport f dépend d'un exposant n qui indique un rapport exponentiel entre le taux de sédimentation et la concentration de matières suspendues dans la zone euphotique, rapport qui se retrouve de plus en plus dans les données empiriques. Des simulations du modèle où l'apport de NO_3 varie temporellement montrent que les pertes de particules peuvent atteindre de 30 à 40 % de la production des systèmes saisonniers oligotrophes. Selon le modèle, $\langle f \rangle$ dans les provinces océaniques à apport limité de N dépend de la variance des bioéléments et de la sensibilité du taux de perte aux variations de la biomasse planctonique dans la zone euphotique.

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Of the total carbon fixed by phytoplankton in the surface layer of the ocean (P_T), only a fraction $\langle f \rangle$ can be removed without destroying the long-term integrity of the pelagic ecosystem; the rest must be recycled in situ (Dugdale and Goering 1967). This quantity $\langle f \rangle \cdot P_T$, called the new production (P_{new}), is the critical component of oceanic primary production limiting the removal of atmospheric CO_2 by marine biota. The new production can be estimated from discrete measurements of the contribution of nitrate to total nitrogen uptake ($f(t)$), if it can be assumed that the nitrate flux represents the supply of N from external sources (Dugdale and Goering 1967). Eppley and Peterson (1979) have shown empirically that $\langle f \rangle$ is nonlinearly related to total primary production, both among and within ocean regions, rising to a maximum of approximately 0.50 for productivity levels exceeding 200 g $C \cdot m^{-2} \cdot yr^{-1}$. The general interpretation of their relationship has been that the losses from the euphotic zone of the oligotrophic open ocean are much smaller (only approximately 10–20% of the organic matter produced) than the losses from productive regions (up to 50%). Accordingly, a value of $\langle f \rangle \approx 0.10$–$0.20$ has been used to extrapolate estimates of respiration in the deep ocean to surface production, implying that production rates in the oligotrophic central ocean are much higher than indicated by the traditional ^{14}C technique (Jenkins 1982; Jenkins and Goldman 1985).

Recently, Platt and Harrison (1985) have arrived at a very different estimate of the particulate losses from at least one open ocean region, the northern Sargasso Sea. Using ^{15}N uptake data reported for two nearshore regions, they found that $f(t)$ could be empirically predicted from the ambient NO_3 concentration. The form of the relationship was very similar to Eppley and Peterson's curve relating $\langle f \rangle$ to P_T, except that $f(t)$ reached a plateau at approximately 0.80. They used this relationship to convert a time series of NO_3 measurements at

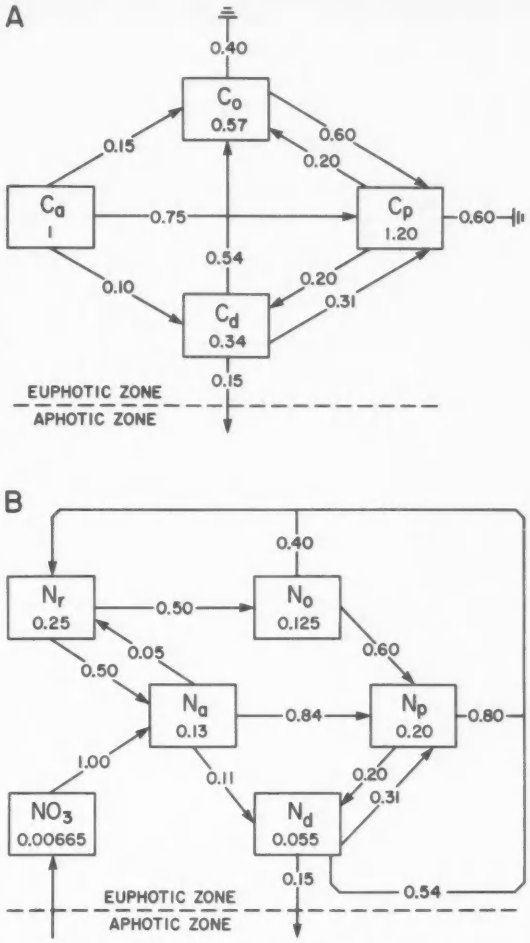

FIG. 1. Compartmental model of the N-limited pelagic food web in the oligotrophic ocean. Compartments: autotrophs (subscript *a*, i.e. C_a or N_a), osmotrophs (*o*), phagotrophs (*p*), detritus (*d*), regenerated nitrogen (N_r), and new nitrogen (NO_3). The flows among compartments are given as proportions of the total flow of C or N (inside the boxes, in $\mu g \cdot L^{-1} \cdot d^{-1}$) through the compartment that originates the flow. (A) Carbon flows; (B) nitrogen flows.

station S in the northern Sargasso Sea into a time series of f ratios. The strong variability in the NO_3 signal implied that $f(t)$ varied from near 0 to 0.80. They calculated a time-averaged $\langle f \rangle$ of 0.32 for this system, two- to three-fold higher than the traditional estimate. With this revised $\langle f \rangle$, they computed an oxygen utilization rate (OUR) from the ^{14}C productivity data that is broadly consistent with that of Jenkins and Goldman (1985). From this perspective, the high OUR's in oligotrophic gyres can be explained by the relatively high losses from the euphotic zone, not by high surface production.

The problem in assessing these conflicting views is that little is known about the short-term variation in $f(t)$ and its relationship to the long-term particulate losses of ocean provinces ($\langle f \rangle$). The data base, especially for the open ocean, is very limited, and the methodology and empirical algorithms are open to criticism. In addition, the reasons for the variability in f ratios are not well understood. The stoichiometry of N fluxes that underlies the interpretation of ^{15}N data does not explicitly consider the time and space scales of the biological processes involved (Eppley et al. 1983). To our knowledge, only Frost (1984) has attempted to model the variation in vertical fluxes in terms of food web dynamics. He found that qualitative changes in the linkages among biological compartments were needed to generate substantial variation in the fraction of the primary production exported. His model, however, is qualitative and treats only fluxes, not standing stocks. Thus, it cannot address the empirical relationships discussed above and the problem of reconciling primary production and particulate fluxes.

In this paper, we develop a compartmental model of N-limited euphotic food webs of the open ocean and use the model to establish relationships between $f(t)$ and $[NO_3]$ and P_T that can be compared with the empirical patterns. We then use simulations of the model to evaluate Platt and Harrison's hypothesis that long-term (i.e. $\geq$1 yr) integrated losses from oligotrophic euphotic zones are significantly higher than previously thought due to short-term variability in new production. The model points to the physical and biotic characteristics of oceanic provinces that are likely to determine the regional variation in the magnitude of particulate losses from surface waters.

Methods

Our procedure was to run numerical experiments in which the NO_3 supply to a simplified food web model (Fig. 1) was changed and selected aspects of its response were recorded and compared. We are particularly interested in the effect on the instantaneous f ratio, the total production, the nitrate concentration, and the instantaneous and time-averaged particulate losses through the base of the euphotic zone. We first describe the model and then the procedures used to experiment with it.

Model of Biogenic Fluxes in the Euphotic Zone

General description

The model subdivides the suspended particulate matter into autotrophs, osmotrophs, phagotrophs, and detritus and distinguishes between new and recycled forms of N (Fig. 1). The mass-specific transfer of C and N among the compartments [T^{-1}] needed to proceed with the numerical experiments were calculated by combining steady-state flow analyses of pelagic food webs (Fig. 1) with current estimates of the distribution of C and N among biological compartments (Table 1). Values for primary productivity and standing stocks were chosen as representative of the low biomass, low production, and high recycling system often associated with subtropical oligotrophic gyres (Eppley 1981). We assumed that ^{14}C productivity estimates for the open ocean were reasonably accurate (Smith 1982; Smith et al. 1984; Williams et al. 1983). Except for vertical losses, discussed in detail below, all fluxes were strictly linear, i.e. directly proportional to the concentration of material in the pool that originates the flux (donor pool). We will examine later the effects of nonlinearities in the C and N fluxes among compartments on the model results.

Vertical particulate fluxes

A number of studies indicate that the sinking flux (S) can be expressed as a continuous function of primary production. As

shown by Betzer et al. (1984), the nearly linear increase in $\langle f \rangle$ over low to moderate productivity levels in Eppley and Peterson's relationship implies that new production ($\approx$ vertical flux) is proportional to the square of total primary production. Betzer et al. (1984) have demonstrated empirically that the regional variation in vertical fluxes of particulate organic C is proportional to production raised to the 1.4 power. Furthermore, the same authors fitted this power function to a time series of particulate fluxes and primary production estimates at station S in the northern Sargasso Sea. Hargrave (1985) confirmed the generality of the nonlinear relationship between vertical flux and production. Bishop et al. (1980) speculated from the lack of coupling between zooplankton standing stocks and primary production in the equatorial Pacific (Blackburn 1973) that vertical flux was nonlinearly related to production, with a higher fraction of the production being lost from the euphotic zone as production increases.

The nonlinearity in the particulate fluxes across the lower boundary of the euphotic zone may be represented in a compartmental model (Fig. 1) by making the loss fluxes from the autotrophic and heterotrophic compartments nonlinear with respect to the standing stocks in those compartments, i.e. for the C flux (S_C):

$$S_C \propto C^n.$$

When $n > 1$, the fractional loss rate $[T^{-1}]$ increases with the concentration of material in the compartment. This simple formulation is advantageous because the sensitivity of the loss constant to variations in concentration can be manipulated through n: the higher n is, the faster the loss constant increases with increases in the standing stock. Conversely, when $n = 1$, the flux is linear with respect to concentration.

Other fluxes

The total primary production, excluding respiration losses, is set at 1 $\mu g\ C \cdot L^{-1} \cdot d^{-1}$ (Dugdale and Goering 1967; Eppley et al. 1977). Most of the production flows to the grazers, with smaller losses to the bacteria (Azam et al. 1983) and detritus. Bacterial production is fueled by dissolved C losses from all the other particulate compartments (Williams 1981). All of the bacterial net production (conversion efficiency = 60%; Joint and Morris 1982) is consumed by the phagotrophs. The phagotrophs are assumed to lose 20% of their gross C intake as dissolved C and 20% to the detritus (Fasham 1985); the remainder is respired. Given these assumptions, the C flow through the detritus is 34% of P_T (Fig. 1); with 15% of that flow lost to the aphotic zone, the vertical C flux is set at 5.1% of the C produced. The downward flux of N is 6.1% of N production due to the stoichiometry of material flows assumed by the model (Table 1).

The pool of regenerated N (N_r) is supplied by the "respired" N and that associated with dissolved organic losses. In the model, the phagotrophs do most of the regeneration (65%), followed by the bacteria (20%), the detritus (12%), and the phytoplankton (3%). This agrees with current appraisals of the sources of regeneration flows in the sea (Harrison 1980). The bacteria compete equally with the phytoplankton for the regenerated N (Laws et al. 1984), while the phytoplankton assimilate all of the new N. The NO_3 uptake was set equal to the N loss rate, or 0.0081 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$ ($f = 0.061$). Since concentrations of inorganic N are generally undetectable in oligotrophic waters (McCarthy 1980), the NO_3 and N_r concentrations were initially set as 0.21 $\mu g\ N \cdot L^{-1}$, halfway between zero and the colorimetric detection limit (Glibert and McCarthy 1984). However, the discrimination against NO_3 uptake (Relative Preference Index (RPI); McCarthy et al. 1977) was much stronger (RPI = 0.1) than generally found (RPI = 0.50–0.9; W. G. Harrison, unpubl. data) and the $[NO_3]$ was adjusted down until a more reasonable RPI was obtained. The resulting $[NO_3]$ is only a few nanomoles, but is actually just below the lower limit of measurements made in the Atlantic with a sensitive chemiluminescent technique (Garside 1985). With these assumptions, the turnover times of the N substrates are 20 h and 4 d for N_r and NO_3, respectively, within the range reported for NH_4 and NO_3 in several oceanic systems (Harrison 1978; Harrison et al. 1983).

TABLE 1. Concentrations of materials (C or N)[a] and C:N ratios[b] of the compartments of a N-limited model of the oligotrophic food web. The C:N ratio given applies to all flows originating from the compartment except when otherwise indicated.

Compartment	Concentration ($\mu g \cdot L^{-1}$)	C:N ratio
C in autotrophs	3.7	6.7[c]
C in osmotrophs	3	4.6
C in phagotrophs	8.1	5.9
C in detritus	7.4	6.1
Regenerated N	0.21	7.52
New N	0.028	7.52

[a]Living C biomasses from Williams (1984); detrital C assumed as 50% of total living C.
[b]C:N ratio of phytoplankton uptake from Eppley et al. (1977).
[c]C:N ratio of phytoplankton excretion is 22.6 (Fasham 1985).

Numerical Experiments

Two types of experiments were run. In one, the rate of NO_3 supply was varied in discrete steps over more than two orders of magnitude (0.0081–3.50 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$) and the steady-state concentrations of C and N in the compartments were evaluated numerically (Nash 1979) for each supply rate. We computed the C and N fluxes as the product of the concentrations and the rate constants and plotted the response of the variables of interest to the changes in the NO_3 flux. We repeated this procedure for models with different values of the exponent that relates particulate losses to concentration in the plankton compartments (n). The loss functions tested vary from linear ($n = 1$) to second order in material concentration ($n = 2$).

In the second type of experiment, we ran the model over several years of simulated time with a continuously varying nitrate supply. The temporal variability in the upward NO_3 flux was simulated using a logarithmically scaled sine curve superposed with a random component (see Fig. 3). The period was 1 yr and the amplitude was adjusted until the simulated range in primary productivity was roughly coincident with that observed at station S (Platt and Harrison 1985). This procedure represented the seasonal cycle of NO_3 upwelling at station S (Menzel and Ryther 1960). The model results were sampled every 3 wk over a period of 5 yr of simulated time. With these simulations, we explored the response of model variables to a fluctuating NO_3 supply and the impact of that variability on long-term C losses from the euphotic zone.

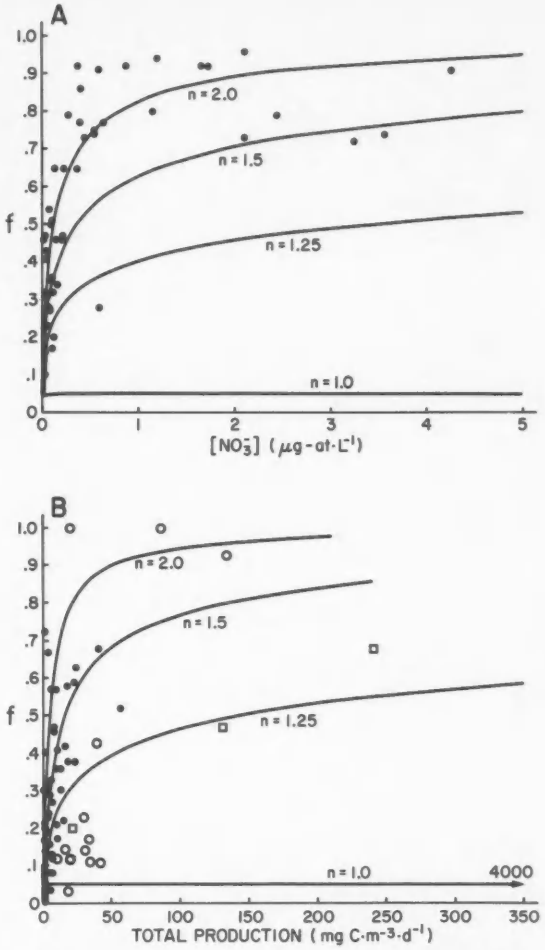

FIG. 2. Relationship of f to (A) ambient nitrate concentration and (B) total production predicted by the compartmental model for different sedimentation functions. At $n = 1$, the rate constant of sedimentation $[T^{-1}]$ is independent of biomass in the autotrophic and heterotrophic pools (sedimentation flux $\propto$ C); for $n = 2$, the sedimentation constant is directly proportional to biomass (flux $\propto C^2$); the other n values represent intermediate levels in the scaling of loss terms to biomass. The data plotted in Fig. 2A are from Platt and Harrison (1985) and Glibert et al. (1982). In Fig. 2B, the solid circles are point estimates from deepwater stations (Dugdale and Goering 1967; Eppley et al. 1977, 1978, 1979; McCarthy and Nevins 1986), the open squares are regional averages (Eppley and Peterson 1979), and the open circles are observations from the middle shelf off Long Island (Yoder et al. 1985).

Results

Steady-State Experiments

The nature of the relationship of $f(t)$ to $[NO_3]$ and P_T depends on the degree of nonlinearity in the particulate losses from the planktonic biota. For $n = 1$, i.e. the percent loss from the plankton is independent of concentration, f at steady state does not change from its initial value in the oligotrophic web (Fig. 2); all material concentrations and fluxes are intensified by the same factor as the nitrate supply. We will show later that this conclusion is not altered substantially by nonlinear phytoplankton uptake kinetics or by a continuously fluctuating NO_3 flux. Linear loss dynamics are inconsistent with the empirical observations of strong variation of $f(t)$ in space and time (Eppley and Peterson 1979; Eppley et al. 1983).

In contrast, setting $n > 1$ leads to theoretical relationships that are much more consistent with the empirical patterns (Fig. 2). Curvilinear relationships between $f(t)$ and $[NO_3]$ similar to the saturating equation of Platt and Harrison (1985) are generated by any value of $n > 1$. The initial slope and the apparent maximum f increase with n. The best fit to Platt and Harrison's data is obtained when $n = 2$. Although there is considerable regional variation in the relationship of $f(t)$ to NO_3, these data are consistent with the relationship for the conditions of low to undetectable ammonia concentration assumed by the model (W. G. Harrison, unpubl. data).

At the same time, the model produces nonlinear relationships between $f(t)$ and P_T of the form published by Eppley and Peterson (1979) (Fig. 2). Again, the steepness of the curves increase with n. The data plotted for comparison are more dispersed than in the case of the $f(t)$ vs. $[NO_3]$ relationships. Most of the data are point estimates of $f(t)$ and P_T made at deepwater stations, and they are roughly bracketed by the theoretical curves for high values of n (1.5–2). The regional averages of Eppley and Peterson (1979) fall along a lower line than the point estimates. We will discuss later the possibility that these averages do not accurately reflect the regional time and space integrated f ratios.

We also plotted data from the middle shelf off Long Island, even though such shallower waters were originally excluded from the Eppley and Peterson model. These data fall into two groups. In one set of observations, made during periods of coastal influence, the f ratios are much lower than would be predicted from both the model and the pattern in the deepwater observations. On the other hand, the f ratios estimated during an oceanic intrusion fall near the $n = 2$ curve. This suggests that the model applies to oceanic water masses irrespective of location and may become a useful tool to model and predict the vertical flux over continental margins where a large fraction of the global C flux in the oceans is hypothesized to occur as a result of nitrate enrichment (Walsh et al. 1981).

Simulation Experiments

The stimulation runs indicated that the results obtained under the assumption of constant NO_3 upwelling rate also apply to systems maintained in a transient state by variability in the NO_3 supply. When losses are linear in concentration ($n = 1$), the effect of the periodic nutrient enrichment is to increase the productivity of the system permanently above its initial level. The amplitude of the seasonal productivity cycles is much reduced over that generally observed in the northern Sargasso Sea (Menzel and Ryther 1960). The mean f ratio is 0.061 (range 0.002–0.28), as predicted from the steady-state model for $n = 1$. Variability in the NO_3 flux increased production, but had little impact on $f(t)$ and none on $\langle f \rangle$. Therefore, instability of the nutrient supply alone is insufficient to explain variations in f among ocean regions (Eppley and Peterson 1979) or depth strata (Knauer et al. 1984; Jenkins and Goldman 1985).

With strongly nonlinear loss dynamics ($n = 2$), the simu-

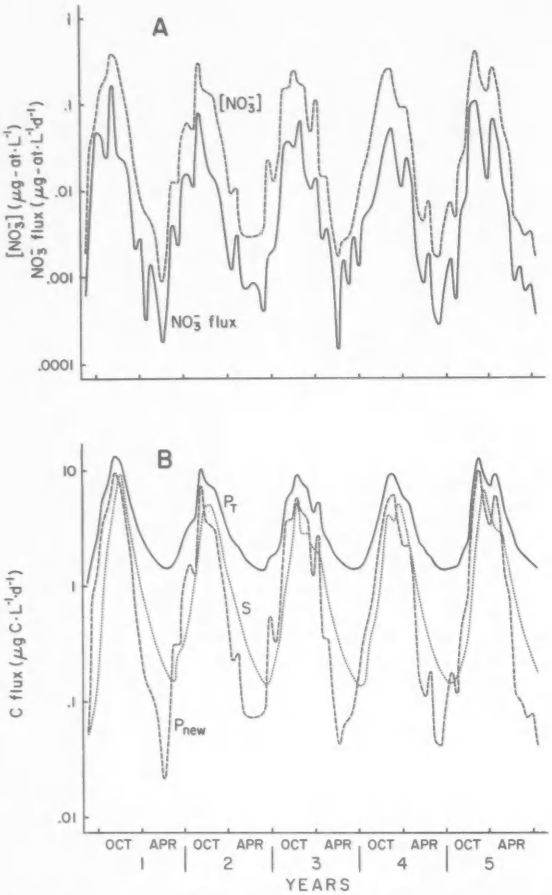

FIG. 3. Time series of (A) upward nitrate flux and mean euphotic zone nitrate concentration and (B) total production (P_T), new production (P_{new}), and downward C flux (S) generated by simulation of the compartmental model ($n = 2$) with a seasonal NO_3 flux. Every 3 d on average, the NO_3 flux was calculated from the sine equation NO_3 flux $= 10^{(-1.17718 + \sin(0.017214 \cdot t))}$ where t is time (days) from the beginning of the simulation and a random disturbance drawn from a lognormal distribution (log variance = 0.5) was added. The flux was then maintained at that value until the next perturbation.

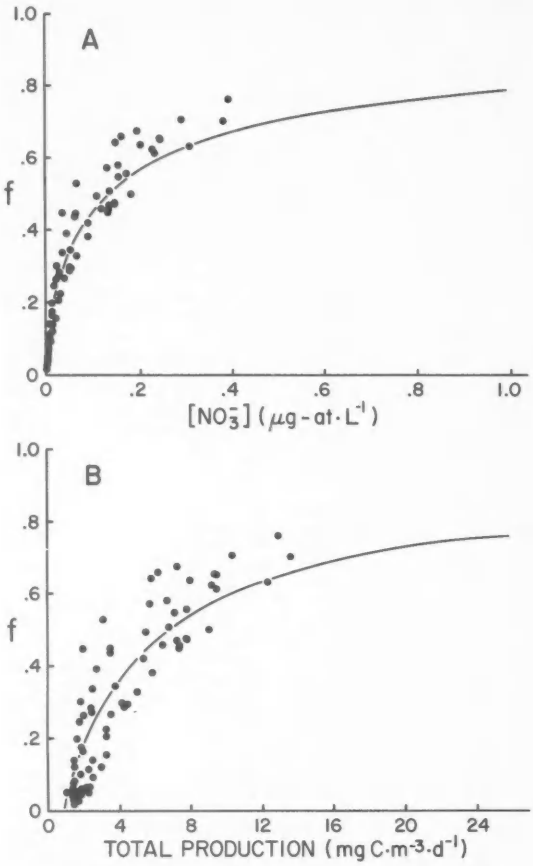

FIG. 4. Covariation of the instantaneous values of f sampled every 3 wk from the time series simulation shown in Fig. 3 with (A) ambient nitrate and (B) total production. The solid curves are the steady-state relationships for $n = 2$ predicted by the model (Fig. 2). Note the change in the scaling of the horizontal axes from Fig. 2.

lated annual cycle of nitrate supply (Fig. 3A) generated seasonal spikes of total production (Fig. 3B) very similar to those observed in the northern Sargasso Sea (Menzel and Ryther 1960). New production accounted for a much larger fraction of the total production during these spikes than during the intervening low production periods (Fig. 3B). The peaks of particulate C fluxes across the base of the euphotic zone lagged behind the P_T and P_{new} peaks (Fig. 3B). The loss rates were higher than indicated by NO_3 uptake during the periods of declining productivity, and the converse for the periods of rising productivity.

The simulated f ratios were tightly related to $[NO_3]$ and followed the steady-state relationship for $n = 2$ (Fig. 4A). The $f(t)$ values also covaried with P_T, except that the wider scatter was not accurately fitted by the steady-state relationship (Fig. 4B). The differences in the fit of f ratios to steady-state curves reflect the differences in the response times of the variables involved. Analysis of the time-dependent behavior of the model showed that the f ratio and $[NO_3]$ approach their steady-state values much more quickly than P_T following a perturbation in the $[NO_3]$ flux. This is not surprising, since the NO_3 dynamics are dependent only on the balance between supply and phytoplankton uptake, while the P_T dynamics are tied to those of the whole food web. This suggests that $[NO_3]$ is a more accurate predictor of $f(t)$ than is P_T in physically dynamic systems.

Estimates of the long-term ($\geq$1 yr) C losses from the model system depend on the way the information contained in the time series is analyzed. The long-term export, $\langle f \rangle$, is calculated as the average of the product $f(t) \cdot P_T(t)$ divided by the average of $P_T(t)$. Since $f(t)$ and $P_T(t)$ are positively correlated, an average of their product will be greater than a product of their averages (Platt and Harrison 1985). The long-term $\langle f \rangle$ calculated as the unweighted average of the simulated f ratios over the 5-yr period was 0.28, which is slightly higher than the f ratio estimated from Eppley and Peterson's curve on the basis of the annual productivity of the northern Sargasso Sea (0.15

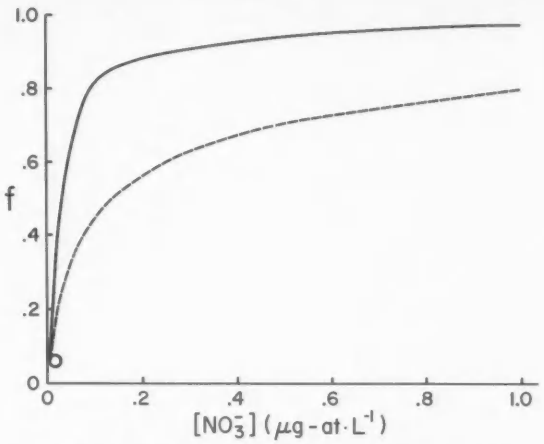

FIG. 5. Relationships of f to ambient nitrate predicted for $n = 1$ (open circle), for $n = 2$ when phytoplankton N uptake is nonlinear (solid curve), and for $n = 2$ and linear N uptake (broken curve). The specific N uptake rate, V (d^{-1}) was described as $V = (V_{max} \cdot N_r)/(K_m + N_r)$ where $V_{max} = 2 \cdot d^{-1}$, $K_m = 0.11\ \mu M$, and N_r is the total dissolved N concentration ($NO_3 + N_r$). The V_{max} was taken as the maximum growth rate of phytoplankton (Banse 1976); the K_m was calculated so that the Michaelis–Menten curve would pass through the V calculated from the uptake fluxes and phytoplankton N assigned to the oligotrophic food web model (Fig. 1; Table 1; $V = 0.275 \cdot d^{-1}$). The specific uptake rates from NO_3 and N_r were computed from V assuming a constant Relative Preference Index (RPI) (W. G. Harrison, unpubl. data) for these N sources. Thus, for NO_3, $V_{NO_3} = RIP_{NO_3} \cdot V \cdot NO_3/N_r$ where RPI_{NO_3} is that calculated from the nitrate concentration and turnover rate assumed for oligotrophic waters.

$< f < 0.26$). But $\langle f \rangle$ computed as the ratio of the cumulative new production to the cumulative total production was 0.42. Estimated as the ratio of cumulative particulate C losses to cumulative production, $\langle f \rangle$ was 0.37. This discrepancy is expected, since the C:N ratio of the detrital fallout is lower than that of production in the model.

Effect of Nonlinear Trophic Interactions

The link between nonlinear loss dynamics and relationships of $f(t)$ to $[NO_3]$ and P_T is robust to nonlinearities in the production and trophic transport of C and N in the euphotic zone. We investigated the effect of nonlinear phytoplankton uptake by assuming that the specific N uptake rate (N uptake normalized to phytoplankton N; Dugdale 1967) was a Michaelis–Menten function of N concentration (Fig. 5). We then solved for the steady-state concentrations and fluxes at each supply rate as before. For $n = 1$, neither f nor $[NO_3]$ changes from its initial value (Fig. 5). This result agrees with the model of Billen et al. (1980) who have shown that microbial populations that follow Michaelis–Menten kinetics effectively maintain a constant concentration of substrate over a range of supply rates, provided that the proportion of the population removed per unit time is independent of population size. With nonlinear loss rates ($n > 1$), the saturating curve relating $f(t)$ to $[NO_3]$ is recovered (Fig. 5). The only difference from the linear uptake situation is that the curve for an equal value of n is shifted to the left, i.e. the f ratio is higher at a given $[NO_3]$ under nonlinear uptake. Nonlinear uptake has no effect on the $f(t)$ vs. P_T curve (not shown here).

Similarly, nonlinear trophic transfers had little impact on our conclusions about the variability of f ratios. We used Lotka–Volterra terms to represent nonlinear grazing fluxes (flux $\propto$ [prey biomass]·[predator biomass]; Landry and Hassett 1982; Smith et al. 1984). With those terms in the model, numerical solutions for steady state proved impossible and we resorted instead to the simulation procedure. With $n = 2$ and the same annual cycle of NO_3 flux as before, nonlinear grazing reduced the cumulative new production and C losses by 15% ($\langle f \rangle$ calculated from C losses fell from 0.37 to 0.32). The curves relating $f(t)$ to $[NO_3]$ and P_T were shifted slightly to the right, i.e. the f ratio is lower at a given value of $[NO_3]$ and P_T under nonlinear grazing. These preliminary investigations suggest a limited impact of nonlinear nutrient uptake and trophodynamics relative to nonlinear losses on the variability of f ratios in natural systems.

Discussion

The Eppley and Peterson (1979) relationship between $\langle f \rangle$ and total production is the accepted description of the variability in the export of primary production among marine ecosystems. Yet, Platt and Harrison (1985) have argued that $\langle f \rangle$ in oligotrophic food webs may have been badly underestimated. Our numerical experiments with a simple compartmental model support the conclusions of Platt and Harrison (1985). Furthermore, the empirical curves relating $f(t)$ to P_T, $f(t)$ to $[NO_3]$, and S to P_T are shown to be expressions of nonlinear sedimentation. With the model, we can explore the relationship between $f(t)$ and $\langle f \rangle$ and the factors that modify that relationship.

The expression of particulate losses as a continuous nonlinear function of the concentration of suspended material is consistent with a variety of mechanisms that promote sedimentation in productive assemblages. The predominance of large diatom cells during blooms or in productive areas is well known (Malone 1971; Semina 1972) and consistent with evidence that large phytoplankters benefit more than small phytoplankters from nutrient enrichment (Parsons and Takahashi 1973; Malone 1980). Not only do large cells settle more rapidly than small ones (Smayda 1970) but their abundance stimulates the large herbivores who are generally considered to be major contributors to the vertical flux through the egestion of fast sinking fecal pellets (Bishop et al. 1977; Knauer et al. 1979). An intriguing possibility is that the increase in particle concentration associated with nutrient enrichment may accelerate the rate of formation of large particle aggregations that can sink very rapidly out of the euphotic zone. There is evidence from a coastal embayment that such a process, which is known to occur in sewage sludge suspensions (Hunt and Pandya 1984), also occurs in the plankton (K. Kranck, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.). If these aggregates are loci for increased microbial and micrograzer activity (Goldman 1984), then the fraction of the community of N regenerators that is carried out of the surface waters would increase with productivity.

The model links the relationships of $f(t)$ to $[NO_3]$ and P_T to the empirical equations that express the C flux as a power function of surface productivity (Betzer et al. 1984; Hargrave 1985). One should not confuse, however, the exponent that relates flux to productivity with that which relates flux to biomass in the model. For $n = 2$, the model predicts that, at steady state, the C flux is approximately proportional to P_T

raised to the 1.8 power. The exponent is slightly lower (1.72) for the time series simulation. In fact, the model does not predict a simple power relation between C flux and P_T, but the theoretical function is nearly linear when plotted against logarithmic axes so that the error incurred by fitting it as a straight line (a power function on untransformed axes) would be very small, and probably undetectable with empirical data.

According to our model, the variation in vertical fluxes in a physically dynamic system can be traced accurately through the [NO_3] field. However, simultaneous indirect (^{15}N uptake) and direct (particulate fluxes) estimates of $f(t)$ may not coincide due to the lag between the initiation of new production in the surface waters and the loss of an equivalent amount of organic matter through the base of the euphotic zone. Of course, this is of no consequence if the objective is to integrate over the whole NO_3 field, as Platt and Harrison (1985) have done. We recognize that various environmental factors, principally ammonia (MacIsaac and Dugdale 1972; McCarthy et al. 1977) and light (MacIsaac and Dugdale 1972), can affect relationships of $f(t)$ to NO_3, but these can be accounted for in empirical algorithms (Platt and Harrison 1985) or in numerical models.

When particle removal is nonlinear, the vertical flux is increased faster than primary production following episodes of nutrient enrichment. Temporal variability in the nitrate fluxes increases the fraction of the C produced that is exported annually to the deep sea. The same argument holds for spatial variability. Therefore, the $\langle f \rangle$ characteristic of oceanic provinces will depend on the variance of the NO_3 field as well as the mean. Undersampling the variance will lead to underestimates of the annual particulate losses.

Another factor that may affect the regional $\langle f \rangle$ is the rate at which particle removal is enhanced by productivity, summarized in this model in the exponent n. Lowering n will increase the production per unit of upwelled nutrient and decrease the losses normalized to surface production. It is possible that regional differences in the structure of the food web affect their response to productivity increases; some webs may incur lesser or greater changes in their structure and particulate losses than others as a result of nutrient enrichment. An example is the difference between coastal and equatorial upwelling systems discussed by Herbland et al. (1985). Or the rate of formation of large aggregates may vary depending on the general hydrodynamic conditions. Particle coagulation is known to be affected by the turbulence of the water column (Hunt 1982). Part of the regional variation in $\langle f \rangle$ may be associated with differences in the sensitivity of the loss rate constant to biomass increases.

Our numerical experiments indicate that variability in the NO_3 flux must be coupled to changes in the fractional removal of the suspended material to generate variability in the f ratios comparable with that observed within and among ocean regions; differences in the stability of the nutrient regime between nearshore and open ocean regions (Eppley et al. 1983) or between the shallow and deep layers of the euphotic zone (Jenkins and Goldman 1985) are not by themselves sufficient to account for that variation. The success of the model in predicting relationships of $f(t)$ to [NO_3] and P_T suggests that other aspects of the productivity and trophodynamics of pelagic ecosystems can be related to nonlinear sedimentation. For now, the model buttresses the argument of Platt and Harrison (1985) that the mismatching of time and space scales between different indices of primary production has led to premature rejection of ^{14}C productivity estimates by some investigators. Conversely, we think that careful consideration of the variability of oceanic systems and of the nonlinear mechanisms of particle loss may lead to revised estimates of the global C flux in the oceans.

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NOTES

Membrane Filtration – Fluorescent Antibody Staining Procedure for Detecting and Quantifying *Renibacterium salmoninarum* in Coelomic Fluid of Chinook Salmon (*Oncorhynchus tshawytscha*)

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We developed a rapid method for detecting and quantifying the pathogen *Renibacterium salmoninarum* in coelomic fluid of spring chinook salmon (*Oncorhynchus tshawytscha*) by concentrating the bacteria on 0.2-μm polycarbonate filters and staining them with specific fluorescein-labeled antibody. Centrifugation of samples and resuspension of the sedimented material in phosphate-buffered saline containing Triton X-100 increased the ease of filtration. Background fluorescence was reduced by counterstaining filters with Eriochrome black T. Postfiltration staining, rinsing, and counterstaining were done in the syringe-mounted filter holders, reducing handling of the filters and possible loss of bacteria. The number of bacteria detected by the filtration – fluorescent antibody technique in a broth culture of *R. salmoninarum* ranged from 6.7×10^7 to 7.6×10^7/mL and was slightly higher than that determined by plate count (9.6×10^6/mL). Increasing the sample dilution or decreasing the number of microscope fields examined generally increased the variability of filter counts of *R. salmoninarum*. Using the filtration – fluorescent antibody technique, we detected the bacterium in the coelomic fluid of 85% of spawning female spring chinook salmon sampled from a hatchery population.

Nous avons mis au point une méthode rapide de détection et de dosage de l'agent pathogène *Renibacterium salmoninarum* dans le fluide du coelome du saumon quinnat du printemps (*Oncorhynchus tshawytscha*) en concentrant la bactérie sur des filtres de polycarbonate 0,2 μm et en les colorant à la fluorescéine avec des anticorps spécifiques marqués. La centrifugation des échantillons et la resuspension du produit sédimenté dans une solution saline tamponnée au phosphate et contenant du Triton X-100, a facilité la filtration. La fluorescence de fond a été atténuée par contre-coloration des filtres avec du noir ériochrome T. La coloration post-filtration, le rinçage et la contre-coloration ont été effectués alors que les échantillons étaient toujours installés dans les porte-membranes fixés aux seringues, ce qui réduit la manipulation des filtres et éventuellement la perte de bactéries. Le nombre de bactéries détectées par cette technique de filtration et d'immunofluorescence dans un bouillon de culture de *R. salmoninarum*, variait entre $6,7 \times 10^7$ et $7,6 \times 10^7$/mL; les résultats étaient légèrement plus élevés que ceux obtenus par numération sur plaque ($9,6 \times 10^6$/mL). Le fait d'augmenter la dilution des échantillons ou de diminuer le nombre de champs microscopiques examinés avait généralement pour effet d'augmenter la variabilité des numérations sur filtre de *R. salmoninarum*. Avec la technique de filtration et d'immunofluorescence, nous avons décelé la présence de la bactérie dans le fluide coelomique de 85 % des femelles du saumon quinnat du printemps prélevées dans une population de pisciculture, qui frayaient.

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Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is a major cause of morbidity and mortality in hatchery-reared salmonids and may have a major adverse effect on ocean survival of these fishes (Fryer and Sanders 1981). Recent evidence indicates that the bacterium is vertically transmitted (from parents to progeny) in association with eggs (Bullock et al. 1978; Evelyn et al. 1984).

We are investigating the relationship between levels of BKD infection in adult spring chinook salmon (*Oncorhynchus tshawytscha*) and vertical transmission of the disease. Quantification of *R. salmoninarum* in coelomic ("ovarian") fluid is of particular interest because of the proximity of the fluid to the

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eggs at spawning and the consequent implications of this for vertical transmission (Evelyn et al. 1984). We considered the existing methods of bacterial quantification by culture to be unsatisfactory for our purpose. Severe culture contamination often results because other organisms infect the debilitated adult spring chinook salmon. Additionally, growth of *R. salmoninarum* is slow; initial isolation often requires 6 wk or longer (Fryer and Sanders 1981). Although rapid tests for detecting *R. salmoninarum* in fish tissues by fluorescent antibody staining have been developed (Bullock and Stuckey 1975; Bullock et al. 1980), these tests are only roughly quantitative and lack sensitivity (Cipriano et al. 1985).

We developed a rapid, simple, and sensitive technique for direct enumeration of *R. salmoninarum* in coelomic fluid by concentrating the bacteria on membrane filters and staining them with fluorescein-labeled antibody produced in goats against the pathogen. We here describe laboratory tests of sensitivity and bacterial count variability for the filtration – fluorescent antibody (FFA) method, and use of the method for detection and quantification of *R. salmoninarum* in spring chinook salmon.

Materials and Methods

For all experiments, each coelomic fluid or broth culture sample (0.5 mL) was diluted with an equal volume of phosphate-buffered physiological saline (PBS, pH 7.1, 0.01 *M* phosphate) containing 0.5% (by volume) of Triton X-100 (PBS–Triton), plus 100 mg thimerosal/L (added as a preservative). After initial dispersion of particulate material by vortex mixing, we centrifuged the sample at 8800 × *g* for 10 min and discarded the supernatant. The pellet was resuspended in 1 mL of PBS–Triton by trituration with a 22-gauge needle fitted to a syringe. Each sample was filtered through a 13-mm-diameter polycarbonate filter (0.2-μm pore size, Nuclepore catalog No. 110406), placed shiny side up, in a Swinney-type syringe-mounted holder (Millipore). A 100-μL volume of fluorescein isothiocyanate (FITC)-conjugated goat anti-*R. salmoninarum* immunoglobulin was placed on each filter. The antiserum (obtained from the Fish Biologics Section, National Fish Health Research Laboratory, U.S. Fish and Wildlife Service, Kearneysville, WV 25430, USA) was filtered through a 0.2 μm Nuclepore polycarbonate filter and then used at the recommended working dilution (1:40). The upper end of each filter holder was closed with Parafilm to prevent evaporation, and the holders were incubated in an upright position at 21°C for 1 h.

After incubation, each filter was rinsed with 3 mL of PBS–Triton and then counterstained with 1 mL of Eriochrome black T (Sigma). The Eriochrome black T was prepared as a 50 mg/L suspension in PBS and filtered through Whatman No. 1 and Whatman No. 42 filter papers before initial use. Both the rinse and counterstain were delivered by syringe. The filters were then removed from the holders, placed on glass microscope slides, and air dried. Cover glasses were mounted with pH 9 buffered glycerol mounting medium (Johnson et al. 1982), with or without 25 mg 1,4-diazabicyclo-[2,2,2,]-octane/mL (DABCO, Sigma), a compound that retards fading of immunofluorescence (Johnson et al. 1982). Filters were examined at 1000× magnification with a Zeiss WL microscope equipped with a Zeiss 4FL epifluorescence illuminator and a Zeiss 487709 filter set for FITC.

We determined the variability of repeated filter counts and the effects of sample dilution and number of microscope fields examined on the counts by using a sample of coelomic fluid pooled from 10 BKD-infected spring chinook salmon from Leavenworth (Washington) National Fish Hatchery (NFH). Serial twofold dilutions (1:2 through 1:16) of the sample were made in PBS–Triton, and 10 filters were prepared for each dilution. Bacteria were counted in 50, 100, and 150 microscope fields on each filter. Filter counts were then converted to cells per millilitre of the original coelomic fluid sample according to the formula of Ecker and Lockhart (1959):

$$\text{Cells/mL} = \frac{\text{(conversion factor) (dilution factor) (no. of cells counted)}}{\text{no. of fields counted}}$$

where the conversion factor is the filtering surface area divided by the area of a single field at the magnification used. The conversion factor for the filter holders, microscope oculars (Tiyoda KW 10×), and objective (Zeiss Neofluar 100×) that we used was 3140 (78.5 mm^2/0.025 mm^2).

The FFA technique was compared with plate counts for detection and quantification of *R. salmoninarum* in a broth culture. The broth form of KDM2 medium (Evelyn et al. 1984) was inoculated with an *R. salmoninarum* isolate (culture identification No. CC-80-369) from Canadian coho salmon (*Oncorhynchus kisutch*) and incubated at 18°C for 7 d. An aliquot of this culture was then used to prepare standard spread plates for colony counts (Koch 1981) in triplicate on modified KDM2 agar medium (Evelyn et al. 1984). The plates were incubated at 18°C for 6 wk. The remaining cells in the broth culture were killed by adding 0.35% formalin (final concentration) and stirring at 4°C for 24 h. Serial 10-fold dilutions of the culture were examined by the FFA method. Total bacteria were counted in 50, 100, and 150 microscope fields on each of three filters per dilution.

In liquid culture medium, *R. salmoninarum* tended to form aggregates. Each clump of bacteria was counted as a single unit because one clump would probably form one colony on the medium used for plate counts.

For field validation, we used the FFA technique to study the distribution of *R. salmoninarum* infection levels in a naturally infected population of sexually mature spring chinook salmon. Coelomic fluid samples were collected from 100 randomly selected female spring chinook salmon from Carson (Washington) NFH. Each sample was taken by expressing a mixture of eggs and coelomic fluid from the oviduct into a paper cup and decanting the fluid into a sterile test tube. A 0.5-mL sample of coelomic fluid from each female was filtered and stained as described above, and bacteria were enumerated in 150 fields on each filter. If initial inspection of a stained filter revealed that bacteria were too numerous to count, 10-fold serial dilutions of the original sample were made in PBS–Triton to reduce bacteria per field to less than 50, and the filtration and staining procedure was repeated.

Results

All steps of the FFA method were usually completed within

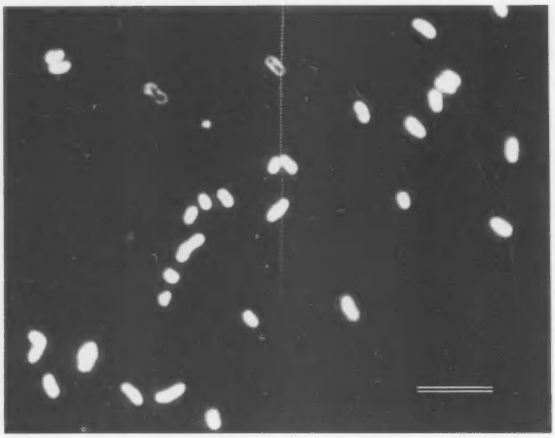

FIG. 1. *Renibacterium salmoninarum* from spring chinook salmon coelomic fluid concentrated on the surface of a Nuclepore 0.2-μm pore size polycarbonate filter. The dark background provided by the Eriochrome black T counterstain contrasts well with the bacteria, which are stained with FITC-conjugated specific antibody. The bar represents 5 μm.

TABLE 1. Variability of *R. salmoninarum* filter counts as a function of coelomic fluid sample dilution and number of microscope fields examined. Ten filters were counted at each dilution. CV = coefficient of variation.

Dilution	No. of fields counted	Mean total no. of cells (SD)	CV (%)	Calculated cells/mL in original culture ($\times 10^5$)
1:2	50	1216 (97)	8.0	3.1
	100	2421 (177)	7.3	3.0
	150	3603 (255)	7.1	3.0
1:4	50	557 (50)	9.0	2.8
	100	1122 (54)	4.8	2.8
	150	1708 (91)	5.3	2.9
1:8	50	306 (51)	16.7	3.1
	100	590 (50)	8.5	3.0
	150	876 (57)	6.5	2.9
1:16	50	118 (21)	18.0	2.4
	100	246 (47)	19.0	2.5
	150	364 (59)	16.2	2.4

2 h for a given sample. The staining and counterstaining procedures resulted in bright specific fluorescent staining of *R. salmoninarum* and a fairly uniform dark background (Fig. 1). The bacteria were easily counted except on filters where they were so numerous (>50 per field) that individual bacteria were difficult to distinguish. Rarely, large fluorescing rod-shaped bacteria and yeasts were observed, but these were morphologically distinct from *R. salmoninarum*.

Estimates by the FFA method of the number of bacteria per millilitre in the pooled coelomic fluid sample used for count variability tests ranged from 2.4×10^5 to 3.1×10^5, depending on the dilution used for counts (Table 1). As expected, serial twofold dilutions of the sample resulted in a reduction in bacterial counts by about half at each dilution. Variability of counts (as indicated by the coefficient of variation) generally increased with increasing dilution. Decreasing the number of fields counted from 150 to 50 also increased variability.

The number of bacteria in the broth culture was calculated to be 9.6×10^6 colony-forming units/mL by plate counts and 6.7×10^7 to 7.6×10^7 bacterial units/mL by the FFA technique, depending on the number of fields counted per filter (Table 2). For both procedures, the highest dilution in which bacteria were detected was 10^{-6}.

In the field validation test, only 15% of the Carson NFH spring chinook salmon tested by the FFA technique were negative for *R. salmoninarum*, although most (65%) of the positive fish had fewer than 10^3 bacteria/mL (Fig. 2). In 7% of the fish, *R. salmoninarum* levels exceeded 10^6/mL; the highest number detected was 1.8×10^8/mL.

Discussion

The FFA technique proved useful for detection and enumeration of *R. salmoninarum* in coelomic fluid because it was rapid and simple to perform and permitted concentration and subsequent detection of small numbers of bacteria in samples. Similar methods have been used for identification and quantification of specific bacteria in water samples (Danielsson 1965; Danielsson and Laurell 1965; Guthrie and Reeder 1969). Our method differed from those previously described in at least two important respects. First, all staining, rinsing, and counterstaining were done while the filters remained in the holders, thus minimizing the handling of the filters and possible loss of bacteria or cross-contamination of filters. Second, our use of mounting medium containing DABCO retarded the fading of immunofluorescence during observation of the filters and permitted storage of filters in a humid chamber at 4°C for at least 2 wk with little loss of fluorescence.

Technical problems forced further modifications of procedures previously reported. Nuclepore polycarbonate filters have been recommended for enumeration of bacteria because the flat surface structure of the filter holds bacteria on a single plane for easy counting (Hobbie et al. 1977). We found, however, that coelomic fluid passed through these filters poorly. The need for filtering the fluid portion of a sample was eliminated by centrifuging the sample, discarding the coelomic fluid supernatant, and then resuspending the pelleted material in PBS–Triton for filtration. Addition of the nonionic detergent Triton X-100 to samples also helped to prevent filter clogging by dispersing particulate material in the samples.

A few samples of coelomic fluid contained so much cellular debris that they passed through filters with difficulty even after Triton X-100 treatment and centrifugation. We recently determined (data not shown) that treating such samples with trypsin according to a procedure modified from that of Pettipher et al. (1980) greatly increased the filterability of the samples without decreasing fluorescent stain quality. A solution of trypsin, prepared by mixing 1 g of trypsin (Difco 1:250) with 100 mL of distilled water at 4°C, was clarified by sequential filtration through Whatman No. 1 and 0.2-μm Nuclepore filters. Each 0.5-mL coelomic fluid sample was mixed with 0.5 mL of PBS–Triton and 0.5 mL of the trypsin solution, heated at 50°C for 10 min, and filtered through a 0.2-μm Nuclepore filter (usually without prior centrifugation). After a rinse with 3 mL of PBS–Triton (delivered by syringe), the filter was stained, rinsed, and counterstained as described above.

TABLE 2. Comparison of FFA and plate count techniques for enumeration of *R. salmoninarum* in a broth culture. A 100-μL volume was inoculated onto each of three plates per dilution, and a 500-μL volume was filtered through each of three filters per dilution.

	Mean bacterial counts (SD)			
		No. of fields counted by FFA method		
Dilution	Plate counts	50	100	150
10^{-4}	96 (35)	60 (3)	110 (7)	159 (10)
10^{-5}	10 (6)	8 (3)	15 (1)	19 (2)
10^{-6}	1 (1)	1 (1)	1 (0)	1 (1)
Calculated count/ mL original culture[a]	9.6×10^6	7.6×10^7	6.9×10^7	6.7×10^7

[a]All calculations were made from the 10^{-4} dilution

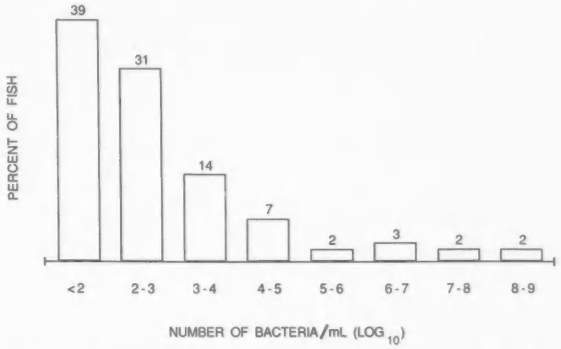

FIG. 2. Distribution of *R. salmoninarum* levels in coelomic fluid of 100 spring chinook salmon sampled from Carson National Fish Hatchery and tested by the FFA technique. The group with less than 10^2 bacteria/mL of coelomic fluid includes 15 fish in which no *R. salmoninarum* cells were detected.

A second technical problem encountered with the FFA procedure was that the Nuclepore filters apparently bound FITC, so that a bright background fluorescence was observed on the filters. Dyeing of filters with substances such as irgalan black (Hobbie et al. 1977), Sudan black B (Zimmermann et al. 1978), or Dylon® (No. 8, Ebony Black; Jones and Simon 1975) is recommended to eliminate this problem. However, these published dyeing methods are time-consuming. Commercially available polycarbonate filters prestained with irgalan black (Nuclepore catalog No. 110456) were tested in preliminary experiments, but these showed no consistent reduction of background fluorescence. The Eriochrome black T counterstaining technique described here provided a rapid and effective alternative to methods previously reported. Eriochrome black T also is used to reduce background fluorescence in cryostat sections and tissue imprints stained with FITC-labeled antibody (Fey 1972).

Repeated filter counts showed the value of increasing the number of microscope fields examined on a filter for reducing the variability of bacterial counts. For the present research, we routinely examined 150 microscope fields (1000× magnification), which was equivalent to about 5% of the total filtering surface area.

The sensitivity of the FFA technique was comparable with that of plate counts for detection and enumeration of *R. salmoninarum* in a KDM2 broth culture. The FFA method was completed much more rapidly than culture (approximately 2 h as opposed to several weeks) and is preferable when results are needed immediately or when severe contamination is likely to occur in cultures. The estimate of bacterial numbers in the original culture obtained by FFA was slightly higher than that obtained by plate counts. The detergent used in our FFA method may have caused better disaggregation of clumps of bacteria, thus explaining the higher counts obtained with this method. The FFA technique also may have detected bacteria that were dead or incapable of growing on KDM2 agar medium. Similar results were reported by Danielsson (1965), who compared FFA counts of *Escherichia coli* with plate counts.

By using the FFA technique, we detected the presence of *R. salmoninarum* in the coelomic fluid of 85% of the adult female Carson NFH spring chinook salmon tested, and individual fish were distinguishable on the basis of numbers of *R. salmoninarum* in the coelomic fluid. The FFA test has a sensitivity nearly equal to that of the enzyme-linked immunosorbent assay (ELISA) and is more sensitive than counterimmunoelectrophoresis, double immunodiffusion, and culture for detection of *R. salmoninarum* in salmonid coelomic fluid (R. J. Pascho, National Fishery Research Center, U.S. Fish and Wildlife Service, Bldg. 204, Naval Station, Seattle, WA 98115, USA, pers. comm.). This method is the first reported fluorescent antibody technique which incorporates concentration of *R. salmoninarum* from fish samples, allowing detection of less than 10^2 bacteria/mL in coelomic fluid.

Implementation of the FFA technique at fish disease diagnostic and research facilities would generally require no expense for equipment, as most such laboratories are already equipped for epifluorescence microscopy. We believe that this technique would be applicable for rapid, nonlethal inspection of spawning salmonid populations for *R. salmoninarum* and for monitoring the effects of various drug treatments or management strategies on *R. salmoninarum* levels in spawning fish.

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Scale Loss and Survival in Smolts of Atlantic Salmon (*Salmo salar*) after Turbine Passage

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Field data obtained from smolts of Atlantic salmon (*Salmo salar*) passed through an Ossberger crossflow turbine indicated that scale loss was greater (24% of the total scaled surface area) in fish that died during the first 48 h after passage than in fish that survived longer than 48 h (16% scale loss) or in controls (15%). Histopathology of selected tissues from turbine-passed fish revealed an incidence of lesions in brain and muscle greater than that detected by gross necropsy. The sensitivity of histologic examination approximately doubled the detection of damage among fish that survived longer than 48 h. Laboratory experiments indicated that an average scale loss of 27% increased mortality in fish held in freshwater. This increased mortality occurred after longer periods (8 d) than in the field, and we suggest that physical damage other than scale loss contributed to earlier mortality in turbine-passed fish.

L'examen sur le terrain de tacons du saumon atlantique (*Salmo salar*) qui avaient traversé une turbine Ossberger à courant transversal a montré que la perte d'écailles était plus importante (24 % de la superficie couverte d'écailles) chez les poissons qui sont morts dans les 48 h qui ont suivi le passage que chez les poissons qui ont survécu plus de 48 h (16 % de perte d'écailles) ou que les témoins (15 %). L'histopathologie de certains tissus prélevés chez des poissons qui avaient franchi la turbine révèle qu'il y a plus de lésions du cerveau et des muscles

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que ce qui est détecté par autopsie sommaire. La sensibilité de l'examen histologique permet de doubler à peu près la détection des lésions chez les poissons qui survivent plus de 48 h. Des expériences en laboratoire ont montré qu'une perte moyenne d'écailles, de l'ordre de 27 %, faisait augmenter la mortalité chez les poissons gardés dans l'eau douce. Cette mortalité accrue s'est manifestée après des périodes plus longues (8 d) que celles sur le terrain, et nous pensons que des dommages physiques autres que la perte d'écailles ont ajouté à la mortalité des premiers jours chez les poissons qui ont franchi la turbine.

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Anadromous fish restoration efforts in the northeastern United States have raised questions about the effect of turbine passage at low-head hydroelectric dams on the survival of outmigrating fishes. One of the questions concerns delayed mortality after turbine passage, specifically the tolerance of fish to the disruption of the skin, scale, and mucus complex and the incidence of mechanical injuries that are not acutely lethal. The importance of the skin, scale, and mucus complex in maintaining the ionic and osmotic integrity in fish is well established (Forster and Berglund 1953; Van Oosten 1957; Potts and Parry 1964; Parry 1966; Koch 1968). However, the degree of disruption (e.g. loss) that can be tolerated is uncertain. Skin damage due to handling and subsequent mortalities has been attributed to ionic and osmotic disturbances (Holiday and Blaxter 1961; Potts and Parry 1964). The removal of scales and mucus decreases the lethal salinity level for smolts of coho salmon (*Oncorhynchus kisutch*) (Bouck and Smith 1979). Although adult rainbow trout (*Salmo gairdneri*) tolerated the loss of up to 25% of scales and mucus in freshwater (Black and Tredwell 1967), Atlantic salmon (*Salmo salar*) larvae died when 1–30% of the skin was removed (Hickey 1979).

Previous quantification of injuries sustained by turbine-passed fish has been limited to differentiating between mechanical or pressure-induced injuries (Cramer and Oligher 1964). These injuries were characterized by gross examination of fish that died during turbine passage. Scale loss in turbine-passed fish has not been quantified. The objectives of this study were to determine the importance of scale loss and physical damage to the mortality of smolts of Atlantic salmon in relation turbine passage.

Materials and Methods

Field experiments — Atlantic salmon smolts from Ossberger turbine studies of Gloss and Wahl (1983) were used to determine (1) the extent of scale loss incurred by fish during turbine passage and (2) whether scale loss and delayed mortality were related. Thirty turbine-passed and 10 reference fish (not turbine passed) were examined. Experimental fish had passed through one of two Ossberger turbines (650 or 850 kW) at various operating modes. However, Gloss and Wahl (1983) found no difference in acute mortality, for fish of similar size, between turbines or among operating modes.

Gloss and Wahl (1983) originally determined mortality of fish up to 48 h after turbine passage. After holding the fish for 48 h, they narcotized survivors with tricaine methanesulfonate (MS-222). Thus, we were able to separate surviving turbine-passed fish into two groups according to survival time: $\leq$48 h and >48 h. Quantification and comparison of scale loss in the three groups was used as an indication of the relation between the extent of scale loss and delayed mortality.

Narcotized or dead fish were placed in individual plastic bags half filled with water and frozen to reduce postmortem scale loss. The fish were later thawed and weighed and the surface area of the scaled portion was determined as described by Webb (1977). Fish were then refrozen to the point of rigidity, eviscerated, beheaded, and halved longitudinally along the dorsoventral midline. Each half was placed flesh-side down on a perforated metal plate. A Plexiglas plate was placed over the scaled side and used to flatten the specimen for examination. Care was taken not to overtighten the apparatus, to avoid distorting of the sections. This apparatus allowed the total scaled surface to lie in the same plane, facilitating quantification of scale loss. Each section was divided into three zones: (1) caudal zone (area above and below the lateral line, posterior to the posterior insertion of the dorsal fin), (2) dorsal region (area just anterior of caudal zone, above the lateral line), and (3) abdominal region (area below the dorsal zone). We used a dissecting scope (15×) and a transparent grid system to determine descaled surface area in each zone.

To determine a mean scale size for each zone, we measured a subsample of 10–20 scales. The square transparent grid pattern approximating the mean scale size was then placed over the appropriate zone. The number of grid intersection points that fell in descaled areas approximated the number of scales missing. The area of descaling was determined by multiplying the number of scales missing by the mean scale size in each of the three zones on both sides of the body, and the percentage of the total surface area that was unscaled was then calculated. Differences in scale loss between groups were determined by applying Student's *t*-test (two-tailed) at the $P < 0.01$ level. Data were square root transformed before statistical treatment.

Laboratory experiments — Scale loss —Two-year-old Atlantic salmon presmolts weighing 45–167 g were obtained from the White River National Fish Hatchery, Bethel, Vermont. Most of the fish had parr marks, were dark in color, and lacked the silver color of fish that have undergone smoltification. All fish had lost some scales; however, only fish with few lost scales were used in the experiments. Holding water, as well as experimental water, was prepared by adding the appropriate amounts of calcium hydroxide, magnesium sulfate, and sodium and potassium chloride to deionized water to yield the following concentration (milligrams per litre) of each of the major ions: potassium and sodium, 1.0; magnesium and calcium, 2.0; chloride, 2.4. Experimental salt water was prepared by adding sodium chloride to the freshwater (17.5 ppt) to simulate ionic and osmotic stress associated with seawater. Fish were acclimated to freshwater and tested at a water temperature of 10°C. They were held in freshwater for 72 h before the experiment was started and fed commercial fish pellets daily ad libitum. Feeding continued after the transfer to salt water.

Five experimental groups of 10 fish each were held for 8 d in

TABLE 1. Mean percent scale loss in different body zones (± SD) of Atlantic salmon smolts after passage through Ossberger turbines.

Survival (h)	N	Scale loss (% total scaled surface area)	Percent of total areas			Percent per unit area		
			Caudal zone	Dorsal zone	Abdominal zone	Caudal zone	Dorsal zone	Abdominal zone
Control	10	15.1±1.9	7.9±1.9	5.4±0.8	1.8±0.5	4.3±0.6	4.1±0.6	1.3±0.4
0–48	13	24.2±4.3	12.1±3.0	7.7±2.4	4.4±1.5	6.5±1.6	5.9±1.6	3.1±0.9
>48	17	15.9±7.3	7.9±3.5	5.6±2.9	2.4±1.7	4.3±1.9	4.3±1.8	1.7±1.2

TABLE 2. Survival and scale removal of Atlantic salmon presmolts (± SD) held 192 h in freshwater followed by 96 h in 17.5 ppt NaCl water. The nominal percentage and area of scale removal for each group was 10% abdominal, 20% abdominal, 30% abdominal, 10% caudal, and no scale removal. N = 10 for each group.

Group[a]	Scales removed (% total scaled area)	Survival (h)	
		Freshwater	17.5% NaCl water
1 (10% abdominal)	8.1±2.0	192.0	57.8±25.5
2 (20% abdominal)	17.7±1.9	191.6±1.3	62.0±22.0[b]
3 (30% abdonimal)	26.6±2.4	164.2±20.1	—
4 (10% caudal)	10.4±1.2	192.0	69.5±26.0
5 (no scale removal)	Controls	192.0	64.4±25.1

[a]Nominal percent and area of scale removal.
[b]N = 9, 1 mortality in freshwater.

freshwater and then transferred and held for 4 d in 50% salt water. This regimen was selected on the basis of downstream migration times and appearance of smolts in estuarine areas (A. Knight, U.S. Fish and Wildlife Service, Laconia, NH 03246, pers. comm.).

All groups (including controls) were anesthetized (MS-222; 1 : 20 000) before scales were removed. Fish from which scales were experimentally removed were then weighed and their scaled surface area estimated. This was accomplished from a regression equation which was developed between weights and areas of scalation in a subsample of fish. A determination was then made as to the scale removal necessary to yield the appropriate percentage. Scales were removed with a dull scalpel blade in a rectangular pattern at the same location on each fish.

Scales were removed in the area below the lateral line and anterior to the posterior insertion of the dorsal ray on both sides of the stomach region in the first and second groups. About 10% of the total scaled surface of the fish was removed in the first group and 20% in the second. In the third group, scales were removed in the same general area as in the first two groups, but also from above the lateral line on both sides of the back; total area of scale removal was 30%. In a fourth group of fish, scales were removed from about 10% of the total scaled surface area, in zones above and below the lateral line, and posterior to the posterior insertion of the dorsal fin, on both sides of the tail region. A fifth group consisted of control fish from which no scales were removed.

Experiments were performed to measure fish tolerance to variable scale loss of about 10, 20, and 30% when the fish were consecutively exposed to fresh and experimental salt water. The number of deaths in each group was recorded daily for the 8-d freshwater exposure. After transfer to experimental salt water, fish were observed hourly for the first 6 h; the interval then gradually increased to 12 h by the end of 96 h, when surviving smolts were sacrificed.

The actual total scaled surface area of fish was then determined. The procedure for scale removal in laboratory experimental fish enabled us to measure scale loss more simply, with a planimeter, than in field experimental fish.

Mechanical injury — Tissue pathology examinations were carried out, on 44 age 1+ turbine-passed Atlantic salmon (mean total length = 164 mm). The sample consisted of 7 fish that had been killed immediately, 6 that died during the first 48 h after passage, and 31 that survived longer than 48 h and were classified as survivors in the experiments by Gloss and Wahl (1983). These 31 specimens were narcotized as the field specimens for scale loss. All fish were preserved in buffered formalin; one opercle was removed and the body cavity was opened by removing the abdominal musculature from one side.

We conducted gross necropsy of external and visceral organs, paying particular attention to the areas where there was visual evidence of injury. Several slices were also made through the somatic musculature. Samples of liver and brain, as well as grossly abnormal tissues, were embedded in paraffin, sectioned at 5–6 μm, and stained with hematoxylin and eosin for microscopic examination.

Results

Field experiments — The average scaled surface of the 40 smolts was 6891 ± 801 (x ± SD) mm^2. The scale loss for the three groups (control, ≤48 h survival and >48 h survival) was calculated as a percentage of the total scaled area, and the relative contribution to the total scale loss by each zone was determined as a percentage of the total scaled surface area for each fish (Table 1).

There was a greater total scale loss in fish surviving ≤48 h than in the other groups. There was no difference in scale loss between controls and fish surviving >48 h. This was also true in caudal and abdominal zones, but there was no difference in scale loss in the dorsal zone among the three groups.

Since the zones differed in relative size (x ± SD; zone 1 = 46.0 ± 1.6%, zone 2 = 23.8 ± 0.9%, and zone 3 = 30.1 ± 1.6% of the total scaled area), the scale loss in each zone was standardized to total scaled area (Table 1) as the percent per unit area zonal scale loss. Thus, the relative importance of each zone was assessed. There was no difference in relative scale loss between the caudal and dorsal zones in any group. However, in all groups more scales were lost from the caudal and dorsal zones than from the abdominal zone.

Laboratory experiments — The proportions of scales removed in each experimental group differed from the nominal values (Table 2). There was no difference in percent scale removal between groups 1 and 4 and no difference in survival due to scale loss in those zones. Only group 3 showed a decrease in survival compared with controls, and this reduced survival occurred in freshwater. There were no significant differences in salt water survival among groups 1, 2, 4, and 5.

TABLE 3 Number and type of injuries detected in gross necropsy and selected tissue histopathology of Atlantic salmon smolts passed through Ossberger crossflow turbines.

Examination and injury	Group: Killed immediately[a]	Survival ≤48 h[a]	Survival >48 h[a]
Gross necropsy			
Hemorrage	6/7	5/6	10/31
Laceration	0/7	1/6	0/31
Contusion	4/7	1/6	10/31
Fracture	0/7	1/6	0/31
Total with lesions	6/7	5/6	15/31
Tissue section			
Brain			
Hemorrhage	4/7	3/6	13/30
Congestion	1/7	4/6	2/30
Total	5/7	6/6	14/30 (8/14)[b]
Muscle			
Hemorrhage	3/3	2/3	6/11
Dystrophy	2/3	2/3	10/11
Total	3/3	3/3	11/11 (0/11)
Liver			
Hemorrhage	0/7	—	1/30
Congestion	0/7	—	4/30
Necrosis	0/7	—	1/30
Total	0/7	0/5	4/30 (3/6)
Total with microscopic Lesions	7/7	6/6	21/31 (9/21)

[a]N detected/N examined.
[b]Increase in injuries detected by microscopic examination when no lesions were evident by necropsy.

No conclusions can be drawn about the relation of scale loss to salinity tolerance in these smolts.

Tissue pathology — Gross lesions consistent with trauma were found in all the Atlantic salmon that died during the first 48 h after turbine passage, in 6 of the 7 that were killed immediately, and in 15 of the 31 that survived for 48 h (Table 3). Microscopic lesions were found in all fish that were sacrificed immediately after turbine passage or that died within 48 h. Brain lesions were frequent in these groups, but no liver lesions were found. Muscle lesions were also frequent in the fish examined, but the prevalence cannot be directly compared with that for brain and liver lesions because the subsample examined microscopically was biased in favor of those having gross lesions.

Microscopic examination added little to the already high frequency of lesions found during gross necropsy in fish which died or were killed. In the fish surviving 48 h, however, gross lesions were present in less than half, and microscopic examination added considerably to the number of affected fish identified. Of the 31 fish examined, 21 had microscopic lesions, and 9 of these were fish without gross lesions. Among these nine fish, the microscopic lesions were identified by examination of the brain of eight and by examination of both the brain and liver in three.

Discussion

There was no difference in average scale loss between turbine-passed smolts that survived >48 h and the controls. However, 43% of the turbine-passed smolts lost 20–28% of their scales, and the percent scale loss was higher in turbine-passed smolts that died within 48 h. The scaleless area in these smolts exceeded that in the controls by 9.1%. Overall variation was large in scale loss among turbine-passed fish (19.4 ± 7.8, $x \pm$ SD, $N = 40$), and high scale loss and high mortality (<48 h) appeared to be related. However, scale loss was probably not the sole cause of death. Smolts with about 27% (group 3) of their scales removed survived 164 ± 20 h in laboratory tests ($x \pm$ SD) in freshwater and none died during the first 48 h. Turbine-passed smolts that died had lost less than 27% of their scales.

About 75% of the Atlantic salmon that survived for 48 h after turbine passage, and nearly all of those that did not survive, had gross or microscopic lesions. Inasmuch as mild, equivocal, or unrelated lesions were not included in these totals, and lesions in survivors were not qualitatively different from those in nonsurvivors, we concluded that many of the survivors in which lesions were found may have died either directly from the injuries or from other problems secondary to the injuries, such as predation or disease. We did not culture the lesions for pathogens, however. As judged by our sample of 31 Atlantic salmon, histological examination would be expected to increase the sensitivity of the necropsy procedure in identifying lesions by about 60%. The brain was the principal organ contributing to that increased sensitivity; the liver added nothing in terms of additional cases identified. If more organs had been routinely sectioned, additional diagnoses would probably have been made on the basis of histopathology. No injuries of the type attributed to pressure changes (Cramer and Oligher 1964) were detected. The hydraulic head at the dam site was 10 m.

Mortality may be due to the disruption of several systems by the mechanical damage that also caused scale loss or to multiple effects in circulatory and respiratory or respiratory and ionosmoregulatory systems. The distribution of scale loss suggested that scales were lost more easily from the tail and dorsal area than from the abdomen. This pattern was not necessarily related to turbine passage or orientation during passage, since it also occurred in the control group. Fish may be more vulnerable to the disruption of ionosmotic homeostasis when scales are lost from the abdominal region, where underlying tissues are more vascular. Muscle may provide a compartmental buffer between the external medium and internal fluids of the organ systems. The tail and dorsal region contain large, thick muscle masses, which may account for a fish's relative tolerance to scale loss in these areas. Bouck and Smith (1979) found that coho salmon were much more sensitive to the loss of scales in the stomach region than elsewhere during a saltwater challenge. Areas most sensitive to scale loss may be the areas least likely to lose scales.

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Simplified Version of the Ampoule–Persulfate Method for Determination of Dissolved Organic Carbon

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A simplified method for the determination of dissolved organic carbon is based upon the ampoule–persulfate wet digestion procedure. The modification proposed involves use of a continuous stream of sparging gas (technical grade oxygen) during the ampoule sealing process to minimize entry of combustion products from the sealing flame. The method provides reliable results with a minimum of training and equipment compared with other methods of sealing ampoules. Further, the proposed method is inexpensive, relatively simple, and compares favorably with costly, automated analysis of dissolved organic carbon.

Le présent document porte sur une méthode simplifiée de détermination du carbone organique dissous, méthode basée sur la technique de digestion en milieu humide du persulfate. La modification proposée comprend l'utilisation d'un jet continu de gaz (oxygène de qualité technique) pendant le processus d'obturation de l'ampoule afin de minimiser l'entrée de produits de la combustion générés par la flamme. On obtient de bons résultats avec un minimum d'expertise et d'équipement quand on compare la présente méthode à d'autres. De plus, elle ne coûte pas cher, est relativement simple et soutient la comparaison avec l'analyse automatisée et dispendieuse du carbone organique dissous.

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Since its introduction over 20 yr ago, the ampoule–persulfate wet digestion method for the determination of dissolved organic carbon (DOC) (Menzel and Vaccaro 1964) has achieved wide acceptance (Strickland and Parsons 1968; American Public Health Association et al. 1975; Wetzel and Likens 1979). The original method (Menzel and Vaccaro 1964) calls for sparging acidified samples with He, quickly plugging the ampoule with silicone grease upon removal of the sparging tube, and sealing the ampoule in a propane–oxygen flame. Advantages of the method include its high sensitivity relative to most thermal combustion methods and the extended storage life of samples after sealing. Disadvantages include the possiblity that a small fraction of total DOC remains unoxidized (Sharp 1973; Baldwin and McAtee 1974; Tate and Meyer 1983) and the technical skill that is needed to properly seal the ampoules (e.g. Froelich 1980).

As originally noted by Menzel and Vaccaro (1964), it is critical that no combustion gases enter the head space of the ampoule as it is sealed. Several authors have proposed methods designed to minimize this problem. Baldwin and McAtee (1974) proposed the use of purge cones which maintain a stream of CO_2-free O_2 as the ampoules are sealed. This mod ification has been incorporated by Oceanography International in the design of a commercially available carbon analyzer (Oceanography International model 524). The purge cone system is cumbersome to use, however, and is frequently omitted by technicians (pers. obs.). Rumyantseva (1977) proposed a method in which the purge tube remains inside the neck of the ampoule as the ampoule is tipped into position for sealing. This method seems quite cumbersome and does not appear to be used to any extent outside the Soviet Union.

In this note, we describe a simple, reliable method for the sealing of ampoules under a continuous gas stream which reults in minimal entry of combustion products as well as atmospheric CO_2 into the ampoule's head space.The general approach which we used was to seal the ampoules under a continuous stream of O_2 using modified electrician's pliers to hold the ampoule and deliver the gas stream. We compared our method with several others, compared the use of O_2 and He in sparging ampoules, and determined the effects of variations in the prescribed protocol. Our method produced data with a precision similar to that obtained by other investigators using methods which require greater care and experience on the part of the operator, as well as more expensive equipment.

Description of Method

Ten millilitres of filtered sample was added to a precombusted ampoule (475–525°C, 4–6 h) containing 50 mg of $K_2S_2O_8$ and 0.2 mL of 0.05 *M* H_2SO_4. Samples were sparged for 15 min with technical (welding) grade O_2 and sealed. Sealing was accomplished by grasping the top of the ampoule with modified electrician's pliers (Fig. 1), holding the lower portion of the ampoule with one's free hand, and placing the neck of the ampoule in a propane–oxygen microburner. Sparging of the ampoule continued during sealing, as O_2 was delivered into the ampoule neck by the fixed cannula attached to the pliers (Fig. 1). During transfer of the ampoule from sparging to sealing, the sparging tube (capillary tube, Kimball 34500) remained in the ampoule head space until the cannula was in place. Thus, the flow of O_2 gas continued, uninterrupted, throughout the sealing procedure.

After sealing, ampoules were autoclaved at 130°C for 1 h. Use of an autoclave with a slow-exhaust cycle, or some other pressure-tight vessel, was imperative. Rapid release of pressure within the autoclave before the ampoules have cooled results in a pressure differential which can cause explosion of the sealed ampoules.

The evolved CO_2 in the ampoules was analyzed using a modified version of the syringe stripping technique (Stainton 1973). Eight millilitres of sample was transferred to a 20-mL syringe using a wide-bore cannula. Twelve millilitres of CO_2-free carrier gas was added to the syringe using a three-way valve, the sample was shaken 50 times to allow for partitioning between liquid and gaseous phases, and 11 mL of gas was injected into the carrier gas stream. Either a nondispersive infrared analyzer or a gas chromatograph (Poropak Q column, TCD detector) was used for analysis of CO_2.

All results are based on analysis of freshwaters following filtration with a glass fiber filter (nominal pore size 0.4–0.9 μm). We use this as our operational definition of "dissolved" organic carbon. No tests of the method were conducted with unfiltered samples (total organic carbon) or seawater, but use of our method of sealing should provide similar advantages for all aqueous samples.

Comparison with Other Methods

We compared the precision of three methods for sealing ampoules. The methods included continuous purging pliers (our method; Fig. 1), the silicone grease method (Menzel and Vaccaro 1964; Strickland and Parsons 1968; Wetzel and Likens 1979), and rapid sealing with neither grease nor continuous purging (Stainton 1973). We compared each method using both zero-grade He and technical-grade O_2 as sparging gases. He, O_2, and N_2 have all been used by previous workers (Table 1). Samples of deionized water (Barnstead Nanopure) and standards containing 3 mg glucose-C·L^{-1} were analyzed using each method.

For both deionized water and glucose standards, we found no significant difference in measured DOC concentrations as a function of sparging gas using the continuous purging pliers ($F = 3381$, df = 1, $p < 0.01$, 16; two-way ANOVA). The three methods of sealing produced different measured DOC concentrations (Table 1). Because of significant ($p < 0.05$) second-order interaction effects (concentration × sealing method × purge gas) found in a three-way ANOVA, we cannot attribute these differences solely to the method of sealing. The concentration of DOC measured with our continuous purging method was both lower and less variable than the other two methods tested (Fig. 2). The higher values obtained by the other methods were probably caused by contamination with combustion products in the flame.

The correspondence between results obtained with reagent-grade He and O_2 as sparging gas shows that any contaminants present in technical-grade O_2 (CO_2 or organics) do not interfere with DOC determinations. Use of a heated cupric oxide catalyst (e.g. Oceanography International model 524) is thus unnecessary. The combination of a simple, reliable sealing method and syringe stripping of CO_2 (Stainton 1973) rather than opening the ampoule by crushing the tip (e.g. Oceanography International) allows us to use two replicates for most analyses, rather than three or four as is commonly used (Sharp 1973; Wetzel and Likens 1979). Very few ampoules are lost during the analytical procedure (about 1–2%).

In addition to preventing the entry of combustion products (Menzel and Vaccaro 1964), maintenance of a continuous

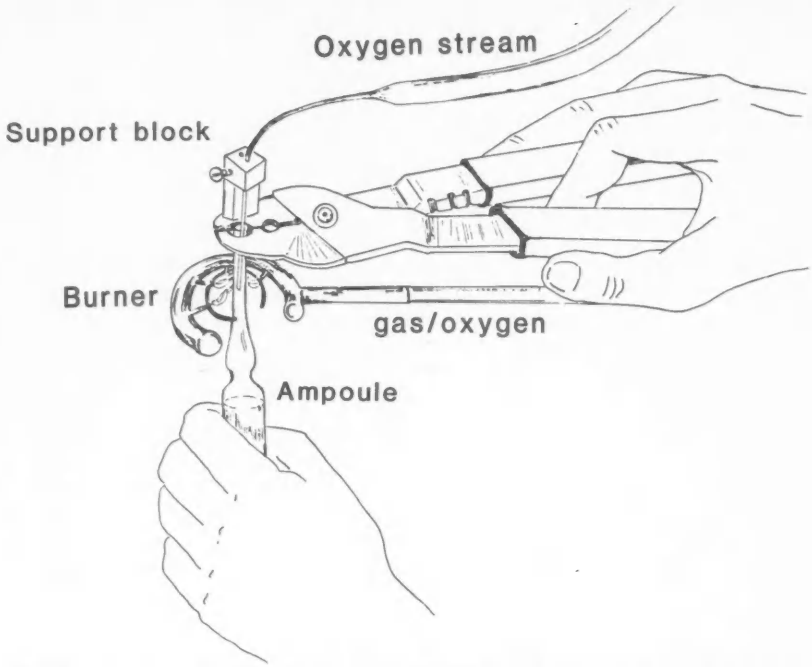

FIG. 1. Sealing of ampoules under a continuous gas stream using the sealing pliers described in this note. Support block is made from cut aluminum with a set screw to hold the cannula. Teflon or other heat-resistant tubing is used to join the cannula to tubing delivering sparging gas.

TABLE 1. Details of various ampoule–persulfate methods for the determination of DOC.

Method	Sparge gas	Sparging time (min)	Sealing method	Deionized water blank ($mg \cdot L^{-1}$)
Menzel and Vaccaro 1964	N_2	3	Grease	0.52
Wetzel and Likens 1979	N_2/He	5	Grease	<0.5
Stainton 1973	He	10	Instant	0.4–0.6
Baldwin and McAtee 1974	O_2	>5	Purge cones	0.3
Rumyantseva 1977	CO_2-free air	15–20	Tipping ampoule	0.48
This study	O_2	15	Continuous sparging	0.20–0.25

stream of sparging gas into the head space of the ampoule during sealing prevents entry of ambient CO_2 (Baldwin and McAtee 1974). Approximately 1.5 mL of head space is found in a sealed 10-mL ampoule containing 10 mL of sample. With an average ambient concentration of approximately 350 ppm CO_2 at STP, 1.5 mL represents 0.28 μg of carbon. Ten millilitres of sample containing 3 mg $C \cdot L^{-1}$ contains 30 μg of carbon. Thus, the entry of atmospheric CO_2 represents an error of approximately 1% for a sample containing 3 mg $DOC \cdot L^{-1}$.

Our method results in lower DOC values for deionized or distilled water blanks than those of many other authors (Table 1). One reason for this is that our method of sealing ampoules results in significantly lower contamination of the head space with combustion products or atmospheric CO_2 than sealing with stopcock grease or rapid sealing of ampoules exposed to the atmosphere (Fig. 2). Menzel and Vaccaro (1964), using both organic-free water and varying volumes of seawater, concluded that the methodological blank was 0.52 mg $DOC \cdot L^{-1}$. Other authors (e.g. Stainton 1973; Sharp 1973) have stated that reagent blanks are negligible and report similar values for distilled water blanks (Table 1).

Blank values greater than baseline can be due to three factors: (1) DOC in distilled or deionized water, (2) organic impurities in reagents, or (3) introduction of CO_2 during the sealing step. Tests for impurities in reagents are relatively simple, and generally this is not a significant problem (Stainton 1973; Sharp 1973). Discrimination between organic contaminants in distilled water and introduction of CO_2 or organics during the sealing process is much more difficult. Our method provides a distinct improvement over previously published ver-

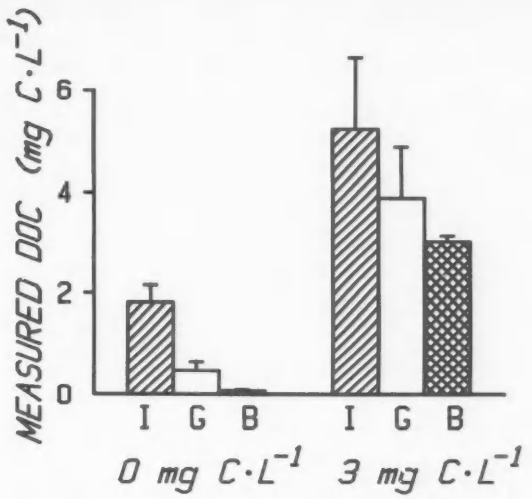

FIG. 2. Comparison of DOC concentrations obtained using three methods of sealing ampoules (I = rapid sealing, B = sealing pliers, G = silicone grease) containing distilled–deionized water or glucose solution (3.0 mg DOC $\cdot L^{-1}$. Bars are means and 95% confidence intervals; $n = 10$. Data using He and O_2 as sparging gas have been averaged.

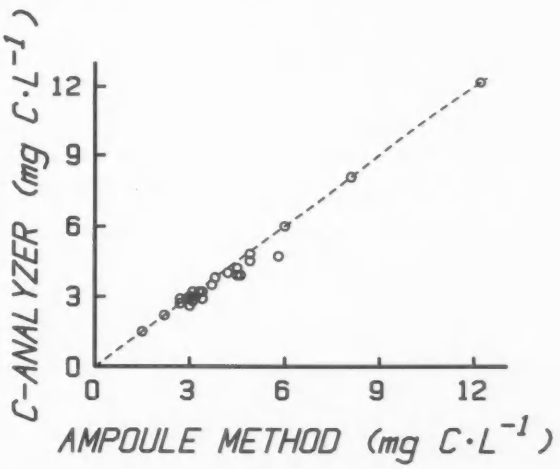

FIG. 3. Comparison of DOC measurements from natural surface water samples using the ampoule method described in this paper (x-axis) and the Dohrmann model 80 UV-catalyzed carbon analyzer (y-axis). Samples were collected from 25 lakes and streams in the Adirondacks (Driscoll and Newton 1985). Drawn line is $y = x$; actual regression is DOC (analyzer) = 0.983(DOC, this method) − 0.104; $r^2 = 0.98$.

sions. Blank values are 0.2 mg $\cdot L^{-1}$ rather than 0.3–0.5 mg $\cdot L^{-1}$ (Table 1). Although we cannot be sure what fraction of this blank is attributable to the analytical method as opposed to contaminated distilled water, we have minimized the uncertainty associated with interpretation of blank values.

We compared the method described here with the automated Dohrmann DC 80, which utilizes a UV-catalyzed persulfate digestion followed by CO_2 analysis on an infrared analyzer. Using surface water samples containing 1–12 mg DOC $\cdot L^{-1}$,

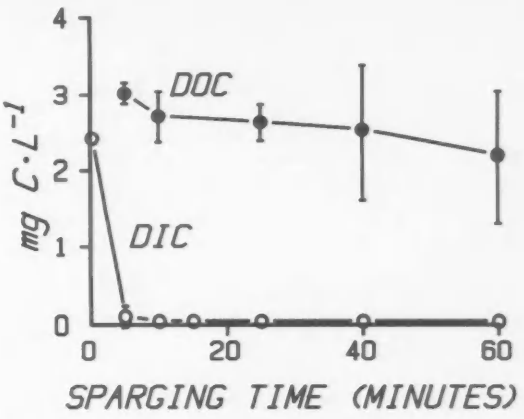

FIG. 4. Concentrations of DOC and DIC as a function of sparging time. Values are means and 95% confidence intervals; $n = 3$.

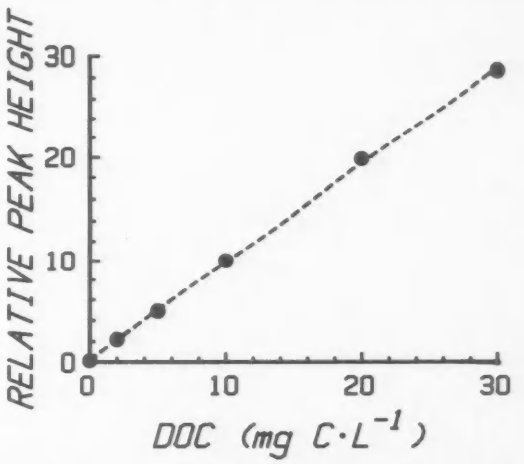

FIG. 5. Standard curve for measurement of DOC (dextrose) using the ampoule–persulfate method described in this note. Samples were analyzed on a Shimadzu GC with injector/detector at 110°C and the column at 50°C. Each point is the mean of from four to eight separate determinations. The mean standard deviation for each DOC concentration was 0.14 peak height units, which is smaller than the size of the plotted points. The average coefficient of variation was 1.98 ± 2.3%. Regression line is $y = 0.959$(DOC) + 0.29; $r^2 = 0.99$.

we found that there was no significant difference in the two methods (Fig. 3). The slope of the regression of DOC (Dohrmann) as a function of DOC (our method) was not significantly different from 1.0 ($t = -0.641$, df = 25, $p < 0.05$), nor was the intercept significantly different from 0 ($t = -0.857$, df = 25, $p < 0.05$). These results show that there was no significant difference in the oxidation efficiency of the two methods, nor was there any significant systematic bias between the two. To compare the precision of DOC analysis by automated UV-catalyzed persulfate digestion and infrared detection of CO_2 (Dohrmann) with our method, we conducted a four-by-four analysis. Four samples collected from Darts Lake in the Adirondacks were each split four ways and the resulting 16 samples were analyzed. Through a two-way ANOVA, we de-

termined the coefficient of variation associated with analytical precision to be 3.2 for the automated Dohrmann unit and 5.4 for the ampoule–persulfate method. While these results suggest that the automated method may slightly improve precision, the two methods give essentially identical results. Our method provides the accuracy of a commercial analyzer without the cost and has the additional advantage that samples can be processed immediately and inexpensively at remote sites for subsequent analysis.

Additional Tests of the Method

We tested the effects of sparging time on sparging efficiency and analytical precision by sparging ampoules for 5, 10, 25, 40, and 60 min. Samples from Mirror Lake, New Hampshire (Likens 1985), were spiked with [^{14}C]$NaHCO_3$; DOC and [^{14}C]-labeled dissolved inorganic carbon (DIC) were measured on three replicates each. ^{14}C-labeled DIC was stripped and counted using the method of Cole and Likens (1979). Ten minutes of sparging was adequate to remove inorganic carbon from the samples (Fig. 4); less than 10 min resulted in significant quantities of DIC remaining in solution. We routinely use 15 min of sparging. Sparging longer than 25 min should be avoided. Variability increased with longer sparging (Fig. 4), probably due to a combination of oxidation of DOC during prolonged sparging and increased likelihood of contamination from airborne particles. Using a one-way ANOVA ($F = 2.10$, $df = 3,8$, $p > 0.178$), we found no significant effect of sparging time on measured DOC concentrations for samples from which DIC was fully stripped (10–60 min total sparging). Thus, we cannot conclude with certainty that oxidation during sparging (as suggested by Sharp 1973) has occurred.

A potential problem with the ampoule–persulfate method is that oxidation may occur before the ampoule has been sealed. Although some oxidation may occur during prolonged sparging (Fig. 4), such oxidation does not appear to occur prior to sparging. We found that filled ampoules containing sample, acid, and persulfate were not affected by a 1.5-h delay prior to sparging and sealing. The DOC concentration of Mirror Lake water used in the sparging time experiment was 2.70 ± 0.14 mg · L^{-1} (mean ± SD $n = 3$) for 10 min of sparging (Fig. 4). After 1.5 h in contact with acid and persulfate, measured DOC was 2.77 ± 0.11 mg · L^{-1} (mean ± SD, $n = 3$).

Our method is generally appropriate for DOC concentrations of 0–30 mg · L^{-1}. For dextrose standards in the range of 0–30 mg C · L^{-1} the response is linear (Fig. 5). This linearity indicates that the persulfate has not been fully reduced by these standards. Thus, for an easily oxidizable substrate in the absence of other reduced compounds (e.g. sulfide) the method will function adequately up to at least 30 mg C · L^{-1}. For a given type of sample, one should demonstrate, by making several dilutions of the same sample, that adequate persulfate is present to fully oxidize the sample. The presence of high DOC and high concentrations of other reduced products, such as in anoxic pore waters, may call for dilution of the sample before sealing.

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Determination of Vitamin B_6 Deficiency in Rainbow Trout (*Salmo gairdneri*) by Liver Enzyme Assay and HPLC Analysis[1]

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Rainbow trout (*Salmo gairdneri*), initially averaging 125 g, were fed a complete or a pyridoxine-deficient diet for 14 wk. Vitamin B_6 status was evaluated biweekly by direct measurement of liver pyridoxine and pyridoxal levels by HPLC and by determining pyridoxal-5′-phosphate-enhanced liver aspartate aminotransferase (ASAT) activity. By 14 wk, mortality had severely reduced the number of fish remaining in the pyridoxine-deficient group. At 14 wk, no significant differences in liver pyridoxine and pyridoxal levels were detected between the trout fed the complete or pyridoxine-deficient diet. Significant differences between dietary groups are found in ASAT activity in liver and percent stimulation of liver ASAT by the addition of pyridoxal-5′-phosphate after 8 wk. Clinical signs of vitamin B_6 deficiency including anorexia, listlessness, frantic and erratic swimming, and ataxia were observed after 11 wk of feeding a pyridoxine-deficient diet. This study shows that vitamin B_6 deficiency in rainbow trout can be readily determined weeks before signs of clinical deficiency are apparent by measuring pyridoxine-enhanced liver ASAT activity. However, liver levels of pyridoxine and pyridoxal are not sensitive indicators of vitamin B_6 status.

Pendant 14 sem, les auteurs ont nourri des truites arc-en-ciel (*Salmo gairdneri*) pesant en moyenne 125 g avec une ration complète, ou dépourvue de pyridoxine. Deux fois par semaine, ils ont déterminé la teneur en vitamine B_6 par quantification directe de la pyridoxine et du pyridoxal hépatiques par chromatographie en phase liquide moderne et par détermination de l'activité de l'aspartate aminotransférase (ASAT) hépatique stimulée par le pyridoxal-5′-phosphate. Après 14 sem, un grand nombre des poissons du groupe nourri avec une ration dépourvue de pyridoxine étaient morts. À ce moment-là, aucune différence significative n'a été observée entre les teneurs en pyridoxine et en pyridoxal hépatiques chez les deux groupes expérimentaux. Par contre, des différences significatives entre ces groupes ont été notées pour ce qui est de l'activité de l'ASAT dans le foie et de la stimulation en pourcentage de l'ASAT hépatique par l'apport de pyridoxal-5′-phosphate après 8 sem. Après 11 sem, les truites nourries avec des rations dépourvues de pyridoxine ont montré des signes cliniques de carence en vitamine B_6 y compris l'anorexie, le manque d'énergie, une activité natatoire frénétique et erratique et l'ataxie. La présente étude révèle que la carence en vitamine B_6 chez la truite arc-en-ciel peut être facilement déterminée plusieurs semaines avant l'apparition de signes cliniques de carence par la quantification de l'activité de l'ASAT hépatique stimulée par la pyridoxine. Par contre, les teneurs en pyridoxine et en pyridoxal dans le foie ne sont pas des indicateurs sensibles de la teneur en vitamine B_6.

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Vitamin B_6 is a required nutrient for all cultured fish. Three compounds possess vitamin B_6 activity, pyridoxine (pyridoxol), pyridoxal, and pyridoxamine, which are converted in the body to the two coenzyme forms pyridoxal-5′-phosphate and pyridoxamine-5′-phosphate (Lehninger 1975). Pyridoxine HCl is the form added to feeds. A dietary deficiency of pyridoxine results in overt signs of deficiency within 4–8 wk in young fishes (National Research Council 1981, 1983). Once pyridoxine (vitamin B_6) deficiency is evident in a group of fish, recovery is uncertain because severely deficient fish do not eat (Halver 1972). Although the depletion of vitamin B_6 forms and coenzymes in the tissues may be a gradual process, the onset of overt signs of the deficiency is abrupt in salmonids fed a pyridoxine-deficient diet and is usually not seen until tissue stores are nearly depleted. Unfortunately, very little time elapses between the first appearance of clinical signs of deficiency and the death of the fish (Halver 1957; Coates and Halver 1958; Smith et al. 1974).

The appearance of clinical signs of vitamin B_6 deficiency in rainbow trout (*Salmo gairdneri*) is preceded by reduced activity of vitamin B_6 dependent enzymes (Smith et al. 1974; Jurss 1978). If the activity of vitamin-dependent enzymes is reduced prior to the appearance of overt clinical signs of vitamin deficiency, it is possible that the absolute levels of the vitamin are also reduced, perhaps in advance of reductions in vitamin-dependent enzyme activity. Direct measurement of the

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TABLE 1. Composition of experimental diets.

Ingredient	g ingredient/kg diet
Vitamin-free casein	400
Gelatin	40
Corn starch	140
Dextrin	92
Glucose	47
Alpha-cellulose	30
Amino acid supplement[a]	20
$Ca_3(PO_4)_2$	48
Vitamin premix[b]	30
Choline chloride, 70% liquid	3
Herring oil	150
Water + mineral premix solution[c]	1000

[a]Amino acid supplement supplied to the diet 0.5% MET, 1.0% ARG, and 0.5% starch.

[b]Vitamin premix supplied to the complete diet (per kilogram of feed): retinol acetate, 7000 IU; vitamin D_3, 3000 IU; DL-alpha-tocopherol, 200 IU; menadione sodium bisulfite, 50 mg; thiamin HCl, 40 mg; riboflavin, 60 mg; D-calcium pantothenate, 200 mg; biotin, 0.5 mg; folic acid, 220 mg; vitamin B_{12}, 0.2 mg; niacin, 300 mg; pyridoxine HCl, 40 mg; inositol, 500 mg; ascorbic acid, 500 mg; choline citrate, 6000 mg. Pyridoxine HCl was omitted from the vitamin premix to produce the pyridoxine-deficient diet.

[c]Mineral premix solution supplied the following per kilogram of diet: $MgSO_4 \cdot 7\ H_2O$, 15 g; NaCl, 7620 mg; KI, 6.55 mg; $CuSO_4$, 8.95 mg; $MnSO_4 \cdot H_2O$, 41.5 mg; $CoCl_3 \cdot 6H_2O$, 20.3 mg; $ZnSO_4 \cdot 7H_2O$, 110 mg; $FeSO_4 \cdot 7H_2O$, 660 mg.

vitamin may be a more sensitive measure of the vitamin status of a fish than measurement of vitamin-dependent enzyme activity. Because of the short time span between the appearance of clinical signs of vitamin B_6 deficiency and death in fish, there is a need for rapid and early detection of a deficiency. This study was thus undertaken to compare the vitamin B_6 status of rainbow trout by the direct measurement of pyridoxine and pyridoxal by high-pressure liquid chromatography (HPLC) and by measuring the activity of a vitamin B_6 dependent enzyme from liver tissue of rainbow trout fed a pyridoxine-deficient diet. Liver was chosen as the sample tissue because of the relatively high concentrations of vitamin B_6 in salmonid liver (Halver 1972).

Materials and Methods

Rainbow trout, averaging 125 g, were randomly distributed from a common group into four 130-L, circular, polypropylene tanks supplied with unfiltered Lake Washington water at a rate of 4–6 L/min. The water temperature was 9°C at the start and 14°C at the end of the study, averaging 11°C. The tanks were indoors, but a natural photoperiod from adjacent windows was observed. Each tank contained 75 fish at the start of the experiment.

The fish were fed to satiation twice daily with one of the two semipurified diets: a complete diet and a pyridoxine-deficient diet (Table 1). Analysis showed pyridoxine levels of 46 and 2.8 mg/kg dry diet in the complete and pyridoxine-deficient diet, respectively. Each diet was fed to two tanks of fish. The diets were mixed and pelleted every 4 wk, and the batches were stored frozen (−12°C) until fed.

At the start of the experiment and then every other week, 10 fish from each treatment were lightly anesthetized with tricaine methanesulfonate (MS-222) and stunned by a blow to the head. The liver was removed, weighed, placed in vials, and frozen at −80°C for later use.

Analytical procedures — Total aspartate aminotransferase (ASAT) activity was determined from liver homogenates as described by D'Apollonia and Anderson (1980) and Casillas et al. (1982). Briefly, liver tissue (100 mg) was homogenized in 7.5 mL of extraction buffer (0.1 *M* sodium phosphate (pH 7.2, 20% glycerol, 0.02% triton X-100, and 1.5 m*M* dithiothreitol). Liver homogenates were then centrifuged at 3000 × g for 5 min. The supernatant was held at 4°C for no longer than 12 h until assayed for enzyme activity. ASAT activity was found to be stable during this period. Measurement of ASAT activity was performed on a Trace III spectrophotometer system (Beckman Instruments, Irvine, CA[2]) at 30°C with or without exogenous pyridoxal-5′-phosphate in the reaction mixture. Absolute ASAT activity per gram of liver tissue and the percent stimulation of ASAT activity in the presence of excess pyridoxal-5′-phosphate were determined.

Liver pyridoxine and pyridoxal determinations by high-pressure liquid chromatography (HPLC) were performed in part by the method first described by McCormick and Wright (1979). Briefly, 600 μg of liver tissue was homogenized in 1 mL of 10% trichloroacetic acid. The homogenates were centrifuged at 3000 × g for 5 min. The supernatant was washed three times with water-saturated diethyl ether. The elimination of primary interfering substances was accomplished by layering the sample onto a mini reverse-phase C18 column (Sep-Pak, Waters Associates, Milford, MA). Pyridoxine and pyridoxal were eluted with a solution of 0.008 *M* 1-heptanesulfonic acid in 10% isopropanol. The portion of the eluate containing pyridoxine and pyridoxal was lyophilized and kept frozen at −80°C until analyzed. The lyophilized product was reconstituted to 300 μL with distilled–deionized H_2O for the analysis.

HPLC analysis of pyridoxine and pyridoxal was performed on a Waters Associates HPLC system (Waters Associates). Reverse-phase analysis was used employing a Bondapak C-18 column (Waters Associates, 3.9 × 300 mm). Elution was performed isocratically using 0.008 *M* 1-heptanesulfonic acid in 10% isopropanol at a flow rate of 1 mL/min and an injection volume of 25 μL. Pyridoxine and pyridoxal were detected at 280 nm (Lambda-Max model 480, LC Spectrophotometer, Waters Associates) and the sample concentration determined by the method of external standards. Addition of pyridoxal and pyridoxine to liver homogenates resulted in recoveries of 58 ± 8% (n = 5) and 73 ± 2%, respectively. Reported pyridoxal and pyridoxine concentrations are not corrected for loss during the procedure.

Data analysis — The data were analyzed by pooled t-test comparing values obtained from fish fed the complete diet with those from fish fed the pyridoxine-deficient diet at each sampling date. A significance level of $P \leq 0.05$ was used.

Results and Discussion

Pyridoxal and pyridoxine levels in the liver of rainbow trout fed a pyridoxine-deficient diet were of no value in detecting a subclinical vitamin B_6 deficiency or confirming a clinical deficiency. Trout fed either a complete or pyridoxine-deficient diet showed no significant difference in liver pyridoxal or pyridox-

[2]Use of tradenames does not constitute endorsement by the National Marine Fisheries Service.

TABLE 2 Pyridoxal and pyridoxine levels (μg/g tissue ± SE) in liver tissue of rainbow trout fed complete or pyridoxine-deficient diets (n = 5 fish per sample).

Weeks	Pyridoxal		Pyridoxine	
	Complete	Deficient	Complete	Deficient
0	2.54 ± 0.26	2.54 ± 0.26	4.01 ± 0.91	4.01 ± 0.91
2	2.63 ± 0.29	2.03 ± 0.51	2.80 ± 0.41	2.75 ± 0.11
4	1.96 ± 0.73	1.33 ± 0.33	1.65 ± 0.15	2.24 ± 0.40
6	1.97 ± 0.12	2.04 ± 0.40	0.77 ± 0.25	1.70 ± 0.55
8	2.04 ± 0.25	1.77 ± 0.20	3.44 ± 0.70	1.45 ± 0.38
10	2.48 ± 0.25	2.10 ± 0.28	1.74 ± 0.22	1.85 ± 0.09
12	2.01 ± 0.36	2.23 ± 0.17	2.27 ± 0.40	2.33 ± 0.23
14	2.22 ± 0.43	2.36 ± 0.42	1.33 ± 0.42	1.66 ± 0.50

TABLE 3. Aspartate aminotransferase (ASAT) activity (units/g wet tissue ± SE) in the presence of pyridoxal-5′-phosphate in liver homogenates of rainbow trout fed complete or pyridoxine-deficient diets (* significant difference ($P < 0.05$) between complete and deficient groups; n = 10 fish per sample).

Weeks	Complete	Deficient
0	112.3 ± 6.3	112.3 ± 6.3
2	80.6 ± 3.0	73.9 ± 4.1
4	86.5 ± 5.1	81.5 ± 10.2
6	108.7 ± 5.3*	112.3 ± 4.7
8	116.3 ± 6.2*	89.2 ± 4.3
10	97.6 ± 6.3*	78.6 ± 4.7
12	103.0 ± 4.0*	70.1 ± 3.5
14	118.3 ± 10.4*	84.5 ± 4.7

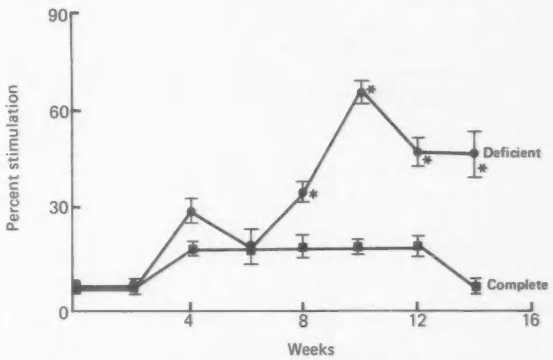

Fig 1. Percent stimulation of ASAT activity (±SE) in liver homogenate of rainbow trout fed complete or pyridoxine-deficient diets. Asterisk indicates significance difference ($P < 0.05$).

ine levels during the 14-wk experimental period (Table 2). Liver levels of pyridoxal and pyridoxine ranged from 2.0 to 2.5 and 1.5 to 4.0 μg/g liver (wet weight basis), respectively, during the feeding trial. Pyridoxamine was detectable but not quantifiable in liver tissue samples. Our results of liver levels are in agreement with Halver (1972) who reported pyridoxine values of 2–3 μg/g liver in salmon fingerlings in hatcheries and 5–6 μg/g liver in adult wild salmon as determined by microbiological assays. Whether direct measurement of vitamin B_6 can provide a useful subclinical and early indicator of vitamin B_6 deficiency may depend on the selection of tissue type. Russell et al. (1985) found that direct measurement of vitamin B_6 from muscle tissues in young pigs fed pyridoxine-deficient diets did not change. They concluded that, in some tissues, some of the vitamin B_6 is bound in a non-exchangable pool and does not necessarily reflect changes in overall vitamin B_6 status. Thus, it is possible that direct measurement of pyridoxine and pyridoxal in liver tissues of rainbow trout may not reflect overall vitamin B_6 status. This does not preclude the possible utility of measuring these parameters in other tissues as a means of providing early subclinical warnings of vitamin B_6 deficiency in rainbow trout.

The fact that our fish fed a pyridoxine-deficient diet were suffering a vitamin B_6 deficiency was evident by changes in liver ASAT acitivity and other clinical signs. ASAT activity in liver of rainbow trout fed the complete or pyridoxine-deficient diets was similar until 8 wk of feeding (Table 3). At 8 wk and thereafter, the ASAT activity of liver was significantly lower ($P \leq 0.05$) in rainbow trout fed the pyridoxine-deficient diet compared with those fed the complete diet. At the onset of clinical signs of pyridoxine deficiency, the ASAT activity was approximately 30% lower in the fish fed the pyridoxine-deficient diet compared with the fish fed the complete diet. The percent stimulation of ASAT acitivity in liver homogenates by the addition of pyridoxal-5′-phosphate became significantly different ($P \leq 0.05$) between dietary groups after 8 wk of feeding and remained so until the end of the experiment (Fig. 1).

Jurss (1978) also observed a significant reduction in ASAT activity in liver in rainbow trout after 4 wk of feeding a pyridoxine-deficient diet. Jurss did not continue his study past 5 wk and thus did not observe signs of clinical deficiency. Our results showing a decrease in ASAT activity in vitamin B_6 deficient rainbow trout confirm those of Jurss (1978) and disagree with those of Smith et al. (1974), who found an increase in liver aminotransferase activity (ALAT) in vitamin B_6 deficient trout. Smith et al. (1974) did not follow changes in ALAT activity during the development of vitamin B_6 deficiency, but only sampled fish that exhibited signs of clinical vitamin B_6 deficiency. Erythrocyte and muscle ALAT acitivities were, however, found to decrease in vitamin B_6 deficient trout. Smith et al. (1974) suggested that the increase in liver ALAT activity was a secondary response to vitamin B_6 deficient induced starvation. Starvation alone was found to increase liver ALAT activity.

Rainbow trout fed the pyridoxine diet ate well and showed no signs of clinical deficiency until after 11 wk of feeding. Between week 11 of feeding and through the termination of the experiment at 14 wk, the fish fed the pyridoxine-deficient diet exhibited signs of vitamin B_6 deficiency. The sequence of signs generally developed initially with anorexia, followed rapidly

by listlessness interrupted by brief episodes of frantic and erratic swimming. Three days after refusing food, individual fish became ataxic and then died within 24 h. Since the exhibition of vitamin B_6 deficiency was spread over 3 wk, at any one time only one or two fish in a tank were affected, making it easy to follow the progression of signs in individual fish.

Smith et al. (1974) observed clinical signs of vitamin B_6 deficiency 8 wk after feeding 150 to 200-g rainbow trout a pyridoxine-deficient diet, whereas 4–6 wk was required for clinical signs to appear in smaller salmonids (Halver 1957; Coates and Halver 1958; Phillips et al. 1958). Growth rates are more rapid in small trout than in large trout (Brett 1979), so tissue nutrients, including vitamin B_6, are more likely to be rapidly depleted than in larger fish. We did not observe rapid gasping and breathing, flexing of opercles, greenish coloration, and tetany before death, which were other clinical signs reported for vitamin B_6 deficient rainbow trout and salmon (Smith et al. 1974; Halver 1972). These unobserved signs are quite distinctive and might be valuable diagnostic guides, but the signs observed in our study were not sufficiently specific to be useful for diagnosing vitamin B_6 deficiency, particularly in a production setting. It is clear from these observations that reliance on clinical signs for determination of abnormalities is neither consistent nor warranted.

The average weights of the fish in the present study were not significantly different ($P > 0.05$) between dietary treatments at each sampling date, although by week 14, the average weight of the fish fed the pyridoxine-deficient diet was 234 g compared with 278 g for the fish fed the complete diet. Smith et al. (1974) reported normal growth during the initial 8 wk of feeding a pyridoxine-deficient diet to rainbow trout; thereafter, growth rates were reduced. The abrupt onset and short duration of overt signs of pyridoxine deficiency also prevent the use of growth rates as a consistent and reliable sign of pyridoxine deficiency.

In summary, our findings indicate that direct measurement of liver pyridoxal and pyridoxine levels by HPLC is not a useful method of detecting or confirming a vitamin B_6 deficiency in rainbow trout. Our findings, however, confirm the value of using ASAT activity to detect vitamin B_6 deficiency in rainbow trout before clinical signs and mortalities occur. Our study also provides ASAT acitivity values that can be used to confirm a suspected vitamin B_6 deficiency in trout exhibiting nondistinctive clinical signs.

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Influence of Alternative Prey Abundance on Pelagic Fish Predation of Larval Fish: A Model

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Pepin, P. 1987. Influence of alternative prey abundance on pelagic fish predation of larval fish: a model. Can. J. Fish. Aquat. Sci. 44: 222–227.

In this model of predation on larval fish by pelagic fish, I postulate that variations in the abundance of the more numerous alternative prey population can determine the time required by a predator to attain its maximum ration. The model predicts that larval fish mortality should be a direct function of the abundance of their predator

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scaled relative to alternative prey abundance. A test of the model using data from the California Current reveals that mortality of larval northern anchovy (*Engraulis mordax*) shows a significant relationship with the predator-to-prey ratio (pelagic fish biomass/zooplankton biomass). Other possible interpretations of this result and problems of model parameterization are discussed.

Dans le présent modèle de prédation des poissons pélagiques sur les poissons larvaires, l'auteur considère que les variations de l'abondance des autres populations de proies plus nombreuses peuvent déterminer le temps requis par un prédateur pour consommer sa ration maximum. Le modèle prédit que la mortalité des poissons larvaires devrait être une fonction directe de l'abondance du prédateur en proportion relative à l'abondance des autres proies. Un essai du modèle réalisé à l'aide de données recueillies dans le courant de Californie révèle que la mortalité des larves d'anchois du Pacifique (*Engraulis mordax*) est en relation significative avec le rapport prédateur–proie (biomasse de poissons pélagiques/biomasse de zooplancton). D'autres interprétations de ce résultat et les problèmes de paramétrage de modèle sont examinés.

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Predation is generally considered a major factor regulating the survival of marine fish larvae (Hunter 1981) and thereby may influence fishery year-class strength. However, predation is difficult to quantify in the field. As a result, the role of predation in regulating larval mortality is not well understood.

A wide variety of invertebrate and vertebrate predators prey upon larval fish. However, planktivorous fish (e.g. herring, mackerel, anchovy) appear to be the dominant predators of larvae beyond the early stages (Lett and Kohler 1976; Winters 1976; Hunter 1981; Skud 1982).

The impact of predation upon larval fish survival is generally examined only in terms of the direct predator–prey interaction. However, adult fish populations are composed of several age-classes and fluctuate on time scales greater than 1 yr. Therefore, it is unlikely that they alone are responsible for the characteristically large interannual variations in year-class strength.

Larval fishes are only a minor component of the plankton (Smith and Lasker 1978; McGowan and Miller 1980) and of the diet of most planktivores (Scott 1920; Savage 1937; Hunter and Kimbrell 1980; Pommeranz 1981). Fluctuations in larval abundance are therefore unlikely to influence strongly the predator's foraging. However, copepods and other zooplankton, which constitute the principal prey of most planktivorous predators, show large variations in abundance interannually as well as over longer and shorter time scales (Colebrook 1978; Chelton et al. 1982; Koslow 1983). This led me to examine whether, based upon simple foraging theory, a significant proportion of the variability in larval survival could arise from the interaction of planktivorous organisms with a variable planktonic food supply.

Pelagic Predator–Prey Model

The Holling disk equation can be used to describe the functional response of predators to prey density (Holling 1959). The daily ration per unit predator (R) (number or biomass of prey eaten per day) is expressed as

$$(1)\quad R = \frac{\lambda N}{1 + \lambda h N} T_f$$

where λ is the rate of successful search per predator foraging time (cubic metres per second), h is handling time (seconds per unit prey, N is prey density (number or biomass per cubic metre), and T_f is the available foraging time (seconds per day). To consider the case of schooling planktivorous fish feeding upon two prey types, larval fish (N_1) and zooplankton (N_2), equation 1 is expanded to a two-species situation (Murdoch 1969):

$$(2)\quad R = \frac{\lambda_1 N_1 + \lambda_2 N_2}{1 + \lambda_1 h_1 N_1 + \lambda_2 h_2 N_2} T_f$$

where subscripts 1 and 2 refer to the parameters associated with larval fish and zooplankton, respectively.

As larval fish are scarce relative to zooplankton ($N_1 <<< N_2$) (Smith and Lasker 1978; McGown and Miller 1980), their influence on the predator's functional response and ration are presumably negligible if $\lambda_2 h_2$ and $\lambda_1 h_1$ are of the same order. Therefore, both terms involving N_1 become insignificant, and equation 2 can be simplied to

$$(3)\quad R = \frac{\lambda_2 N_2}{1 + \lambda_2 h_2 N_2} T_f.$$

We can rearrange equation 3 to obtain

$$(4)\quad T_f = \frac{R(1 + \lambda_2 h_2 N_2)}{\lambda_2 N_2}.$$

This states that if ration level is constant, foraging time is primarily determined by the abundance of zooplankton prey (N_2). Presumably, larval fish are preyed upon in proportion to encounter frequency. The total impact upon the fish larvae by the predator can then be expressed as

$$(5)\quad R_1 = \frac{\lambda_1 N_1}{1 + \lambda_2 h_2 N_2} T_f P$$

where R_1 is the number or biomass of larval fish eaten per day per cubic metre and P is predator density. The parameters for zooplankton are used in the denominator because as the most abundant prey type, it will ultimately limit the predator's instantaneous ability to handle prey. Substituting the value of T_f from equation 4, we obtain

$$(6)\quad \frac{R_1}{N_1} = \frac{\lambda_1 R P}{\lambda_2 N_2}.$$

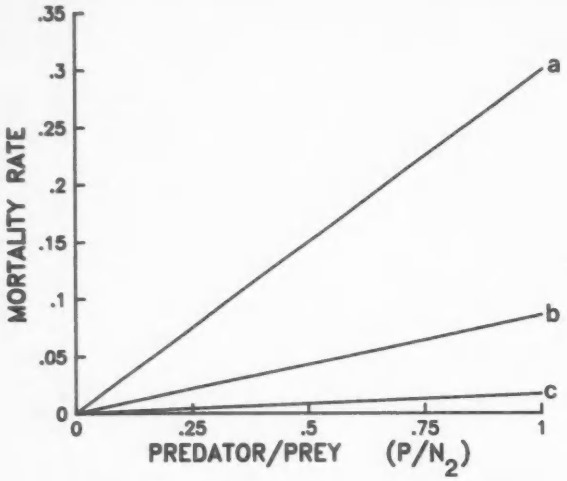

FIG. 1. Coefficient of instantaneous daily mortality (R_1/N_1) as a function fo predator-to-prey ratio (P/N_2). Predator ration (R) is 5% body weight $\cdot d^{-1}$. Values of successful rates of prey encounter ($L \cdot min^{-1} \cdot g^{-1}$) for fish larvae ($\lambda_1$) and zooplankton ($\lambda_2$), respectively, are (a) 0.24, 0.04), (b) 0.15, 0.087), and (c) 0.05, 0.15). These values provide maximum range of ratios of the rates of successful encounter characteristic of Atlantic mackerel (see text).

This relationship states that larval mortality is a function of predator abundance scaled relative to planktonic prey availability.

I parameterized equation 6 to examine whether larval survival may be realistically considered to be influenced by the interaction of planktivorous fish and alternative planktonic prey. The average rate of successful search by schooling Atlantic mackerel (*Scomber scombrus*) for fish larvae between 2 and 10 mm in length (λ_1) and zooplankton (λ_2), estimated from laboratory experiments, are, respectively, 0.15 $L \cdot min^{-1} \cdot g$ $mackerel^{-1}$ (range 0.05–0.24) and 0.087 $L \cdot min^{-1} \cdot g$ $mackerel^{-1}$ (range 0.04–0.15) (Pepin 1985). These values reflect measurements taken with fish swimming at approximately 30–32 $cm \cdot s^{-1}$ (1 body length $\cdot s^{-1}$). They may be reasonable for other species such as herring but may be high for smaller species such as pilchards and anchovies. For this analysis the predator was assumed to become satiated at a ration of 5% body weight $\cdot d^{-1}$ (Mathisen et al. 1978; Hunter and Leong 1981). Within the range (0.5–1.0) of predator-to-prey biomass ratios suggested by Sheldon et al. (1972, 1977) and Silvert and Platt (1980), larval fish daily mortality rates range from 5 to 22% $\cdot d^{-1}$ (Fig. 1), which is of the order of observed larval mortality rates (Theilacker and Dorsey 1980).

Model Verification

To test model predictions, large data sets of larval fish mortality, pelagic planktivorous fish abundance, and zooplankton biomass are required. Observations on the California Current ecosystem (MacCall 1979; Zweifel and Smith 1981; Smith and Eppley 1982) were used in this analysis.

Two times series of larval fish mortality were available for the central subpopulation of northern anchovy (*Engraulis mordax*). One data set consisted of quarterly estimates of larval mortality from a single inshore stratum of the CalCOFI grid within the population center (1951–66, Zweifel and Smith 1981). These estimates of larval mortality rates were calculated for larvae between 3 and 15 mm in length and reported as a mortality coefficient per millimetre. I used estimates for the periods of January–March and April–June, the periods of peak larval anchovy abundance (Smith and Eppley 1982). The second data set consisted of larval mortality estimates for the period of January–April (1951–66) for the entire northern anchovy central subpopulation (Lo 1983). These larval mortality rates were estimated for larvae between 3 and 8 mm in length and reported as a cumulative mortality coefficient for larvae between 3 and 8 mm. The purpose for using two different time series was twofold. First, mortality rate estimates from Zweifel and Smith's (1981) study were within the population center and may therefore have been less subject to environmental factors such as advection which may be more important along the periphery of a population's range. Second, estimates of larval mortality rates from Zweifel and Smith (1981) were averaged over a broader larval size range than the mortality rates calculated by Lo (1983). Since predation on larval fish by pelagic fish is size selective (Pepin 1985), the impact of pelagic fish and the influence of alternative prey may be more readily detected using mortality rates estimated over a broader range of larval anchovy lengths. Each data set was used independently to test the model.

The time series for quarterly zooplankton biomass (grams per square metre) are taken from Smith and Eppley (1982) who recalculated Longhurst et al.'s (1972) measurements of displacement volume from surveys conducted using the CalCOFI 550-μm-mesh net. This plankton time series is coincident with the region in which larval anchovy mortality rates were estimated. However, quarterly values from this time series were centered around the months of March (February–April) and June (May–July) in contrast with data of larval anchovy mortality which are centered around the months of February and May for Zweifel and Smith's (1981) data. Furthermore, Lo's (1983) estimates of larval anchovy mortality rates are calculated over a longer time period than the estimates of zooplankton abundance for the quarter of February–April. Although these discrepancies may introduce some error into the analysis, changes in alternative prey availability should be adequately represented by these data.

Pelagic fish biomass was taken as the combined biomass of adult northern anchovy in the California Bight subpopulation (Smith and Eppley 1982) and the biomass of 2+ Pacific sardine (*Sardinops sagax*) as estimated by MacCall (1979). Both these species occur in the southern California Bight during January–June (Culley 1971; Smith and Eppley 1982). All estimates were converted to grams per square metre by dividing the estimated adult biomass by 3×10^{10} m^2, the area of the southern California Bight used by Smith and Eppley (1982).

Analysis consisted of performing linear regressions using larval anchovy mortality as a dependent variable with any single factor (pelagic fish biomass (P), zooplankton abundance (N_2), and the predator-to-prey ratio (P/N_2)) as the independent variable. A comparison of the F-values and the coefficients of determination (r^2) allows us to assess whether the predator-to-prey ratio shows a better relationship with larval mortality than either of its components. This was necessary because the direct interaction between larval fish and their predators or zooplankton, which may serve as prey for the fish larvae, may be a better predictor of larval mortality.

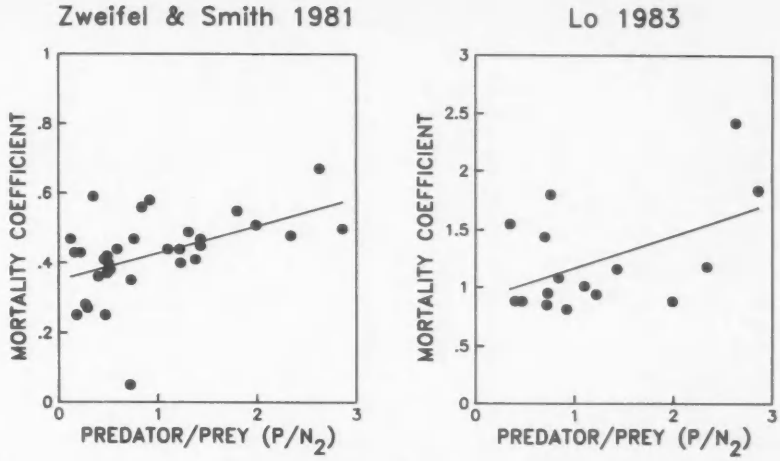

FIG. 2. Field mortality rates of larval northern anchovy, estimated by Zweifel and Smith (1981) for stratum No. 7 of the CalCOFI survey grid (left-hand panel) and by Lo (1983) for eight regions of the CalCOFI survey grid (4, 5, 7, 8, 9, 11, 13, 14) enclosing the central subpopulation (right-hand panel), as a function of the predator-to-prey ratio (pelagic fish biomass/zooplankton biomass) for the California Current region. The lines represent the relationships (left-hand panel) $Y = 0.33 + 0.0057X$ and (right-hand panel) $Y = 0.88 + 0.28X$.

The results of the analysis using Zweifel and Smith's (1981) data show that larval anchovy mortality has a stronger relationship with the predator-to-prey ratio ($Y = 0.35 + 0.080X$, $F = 9.57$, $r = 0.50$, $n = 32$, $P < 0.01$) (Fig. 2), which accounts for 25% of the variance, than either pelagic fish biomass ($Y = 0.33 + 0.0057X$, $F = 6.43$, $r = 0.42$, $n = 32$, $P < 0.05$), which accounts for 18% of the variance, or zooplankton abundance ($r = -0.21$, $n = 32$, $P > 0.05$), which accounts for only 4% of the variance.

Analysis of Lo's (1983) data shows that for larval mortality rates estimated for a broader geographic area and for a smaller size range of larvae, the predator-to-prey ratio provides a slightly better relationship ($Y = 0.88 + 0.28X$, $F = 4.59$, $r = 0.50$, $n = 16$, $P = 0.05$) (Fig. 2) than predator biomass alone ($Y = 0.84 + 0.24X$, $F = 3.92$, $r = 0.46$, $n = 16$, $P = 0.07$). Zooplankton abundance was not significantly correlated with Lo's estimates of larval mortality ($r = 0.13$, $P > 0.20$).

The predator-to-prey ratios estimated in this analysis are much higher (max = 2.8) than those used to parameterize the model (max = 1.0). However, this may be due to undersampling small zooplankton as a result of the large mesh size (550 μm) used in the surveys and/or to calculating fish population densities over too restricted an area. Therefore, although I use the analysis as confirmation of the model's prediction, I place little importance on the values for the slopes of the relationships between larval mortality and the predator-to-prey ratio.

Discussion

The critical importance of availability of alternative prey in influencing predation upon young fish has been previously suggested by a number of researchers (Nielsen 1980; Hopkins et al. 1982). Although I parameterized the model to estimate the impact of predation by pelagic fish, the availability of alternative zooplankton prey should also affect predation upon larval fish by invertebrate carnivores that exhibit a Holling type II functional response and feed on both zooplankton and fish larvae. According to equation 6, the impact of each predator type is dependent on the relative biomasses of that predator and its alternative prey as well as the predator's metabolic requirements. The ratio of successful predator search rates for larval fish relative to zooplankton, which may also be an indicator of prey preference, only determines the sensitivity of predator-induced larval mortality rates to fluctuations in zooplankton abundance because I assume a constant ration level (equation 4). Therefore, predation on larval fish by pelagic fish may be more sensitive to changes in zooplankton abundance than predation on larval fish by invertebrate carnivores, since the former show a greater relative preference for fish larvae (Kuhlmann 1977; Pepin 1985).

However, three assumptions, implicit in the parameterization, may limit the model's generality. First, predator-induced mortality rates are calculated assuming a homogeneous environment. However, marine organisms are patchily distributed (Steele 1976). Frank and Leggett (1985) showed that abundance of fish larvae is negatively correlated with macroinvertebrate predator abundance. They purposed that this relationship may reflect an adaptive response on the part of fish populations to release their larvae in predator-poor environments rather than a depletion of larvae by the predators. Furthermore, Daan et al. (1985) suggested that the spatial distribution of adult herring age groups in the North Sea significantly influences their impact on cod and plaice eggs. Therefore, the degree of spatial and/or temporal overlap between predator and both prey populations will influence the importance of alternative prey abundance on the predator's impact.

Second, I assume in equation 6 that the paramters R, λ_1, and λ_2 are constant. However, current evidence suggests that predator ration, foraging velocity, and prey preference are influenced by the size, species composition, and total abundance of their prey. Unless parameter changes are proportional in both the numerator and denominator of equation 6, larval fish mor-

tality will no longer be a linear function of P/N_2. In such cases, relative preference would no longer be constant for different levels of prey abundance. Therefore, predator responses such as switching would reduce the usefulness of the predator-to-prey ratio as a measure of the impact of predation on larval fish survival.

Third, I assume that the predator will not influence the alternative prey population. However, changes in predator abundance may alter zooplankton community structure (Brooks and Dodson 1965). This may result in changing the mean rate of successful search for alternative prey (λ_2) and thus alter the functional relationship in equation 6. However, Koslow (1983) concluded that changes in the pelagic zooplankton community of the northeastern Atlantic do not appear to be regulated by changes in the abundance of herring and mackerel stocks.

Despite these three potential limitations, the relationship between mortality rates of larval northern anchovy and the predator-to-prey ratio for the California Current region (Fig. 2) is significant for both Zweifel and Smith's (1981) and Lo's (1983) data. However, these relationships may only reflect general environmental conditions because numerous other biological and physical factors, many of which are strongly correlated (e.g. southward transport and zooplankton; Chelton et al. 1982), may be influencing larval fish mortality (e.g. offshore transport).

In addition to the limitations outlined above, the model fails to incorporate the direct influence of zooplankton abundance on larval fish survival either through growth, which may influence the time spent within the predatory field, or starvation. In fact, Shepherd and Cushing (1981) concluded that larval fish mortality may be proportional to P/N_2 (their equation 12) by assuming that zooplankton influence larval fish growth but not predator foraging. Therefore, the results of my analysis may include the influence of zooplankton abundance on larval survival through either starvation, growth, predator foraging time, or a combination thereof.

Because of the additional factors discussed above, the analysis of the California Current anchovy, although consistent with the model, was only preliminary. A number of other factors may be involved and the mechanisms underlying the relationship between zooplankton abundance and larval fish mortality must be clarified. Future investigations should focus on the interaction between larval food availability and predation to determine if both influence the same proportion of the larval population.

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Acute Toxicity of Methoxychlor to Larval White Sucker (*Catostomus commersoni*) as Modified by Age, Food Availability, and Egg Preexposure

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Holdway, D. A., E. J. Kempe, and D. G. Dixon. 1987. Acute toxicity of methoxychlor to larval white sucker (*Catostomus commersoni*) as modified by age, food availability, and egg preexposure. Can. J. Fish. Aquat. Sci. 44: 227–232.

Methoxychlor is currently pulsed into several western Canadian rivers at 0.30 mg·L^{-1} to control the emergence of blackflies (Diptera: Simuliidae). A 2-h exposure of white sucker (*Catostomus commersoni*)) eggs to 4.83 mg methoxychlor · L^{-1} at 1, 3, or 6 d postfertilization increased the incidence of deformities in both 1-d-old and pooled 3- and 6-d-old eggs (2.9 and 3.1%, respectively) compared with control eggs (1.1%). Larvae from exposed eggs showed a 2-d delay in the onset of exogenous feeding. Exposure as eggs increased methoxychlor tolerance. Respective 168-h PE LC50s (the 2-h pulse-exposure concentration causing 50% mortality over 168 h) for 2-d-old larvae from control eggs and eggs exposed for 2 h to methoxychlor at 1, 3, or 6 d postfertilization were 0.36, 1.04, 2.05, and 2.67 mg·L^{-1}. Methoxychlor toxicity in control larvae was modified by age; 10-d-old larvae were less tolerant than 2-, 18-, and 26-d-old larve, with respective PE LC50s of 0.04, 0.36, 0.34, and 0.20 mg · L^{-1}. After the onset of feeding, food availability modified toxicity. The 96-h PE LC50s for 18-d-old fed and unfed larvae and 26-d-old fed and unfed larvae were 0.34, 0.032, 0.20, and 0.038 mg · L^{-1}, respectively. Swimbladder development was arrested in 2-d-old larvae exposed for 2 h to 0.10 mg methoxychlor · L^{-1} at 9 d of age. White sucker survival may be at risk in methoxychlor-treated rivers.

Du méthoxychlore est actuellement déversé dans les eaux de plusieurs cours d'eau de l'Ouest canadien au taux de 0,30 mg·L^{-1} dans le cadre de la lutte contre la mouche noire (Diptera : Simuliidae). Une exposition de 2 h d'oeufs de meunier noir (*Catostomus commersoni*) à une concentration de 4,83 mg·L^{-1} de méthoxychlore (1,3 et 6 d après la fertilisation) a entraîné une augmentation de l'incidence de difformités chez les oeufs de 1 d et les oeufs groupés de 3 et 6 d (2,9 et 3,1 % respectivement) par rapport au groupe témoin (1,1 %). Chez les larves provenant d'oeufs exposés, l'alimentation exogène a été retardée de deux jours. Une exposition à l'état d'oeuf a accru la tolérance au méthoxychlore. Chez des larves de 2 d provenant d'oeufs témoins et d'oeufs exposés pendant 2 h au méthoxychlore 1, 3 et 6 d après la fertilisation, la PE LC50 (la concentration qui après 2 h d'exposition pulsée entraîne la mort de 50 % des individus après 168 h) s'élevait respectivement à 0,36, 1,04, 2,05 et 2,67 mg·L^{-1}. La toxicité du méthoxychlore variait selon l'âge chez les larves témoins; ainsi, les larves de 10 d étaient moins tolérantes que celles de 2, 18 et 26 d, avec des PE LC50 respectives de 0,04, 0,36, 0,34 et 0,20 mg·L^{-1}. Après le début de l'alimentation, la disponibilité de la nourriture a modifié la toxicité. Chez les larves de 18 d et de 26 d nourries et à jeun, la PE LC50 après 96 h s'élevait respectivement à 0,34, 0,032, 0,20 et 0,038 mg·L^{-1}. Le développement de la vessie natatoire s'est arrêté chez les larves de 2 d exposées pendant 2 h à 0,10 mg·L^{-1} de méthoxychlore, et chez les larves de 39 d exposées pendant 2 h à la même concentration après 9 d d'existence, la croissance était réduite. Ainsi, la survie du meunier noir peut être menacée dans les cours d'eau traités au méthoxychlore.

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Methoxychlor has been used to control emergence of blackfly larvae (Diptera: Simuliidae) in western Canada since 1974, principally in the Athabasca and Saskatchewan River systems. Treatment involves the introduction of a 25% emulsifiable concentrate into the rivers to create a pulse of methoxychlor (0.30 mg·L^{-1} nominal) which is carried downstream by the current. Although nominal exposure times range from 7.5 to 15 min, nonlaminar flow, adsorption, and precipitation result in longer exposures (NRC 1983).

Eggs of the white sucker (*Catostomus commersoni*) are deposited and hatch from May to June (Scott and Crossman 1973), coincident with larviciding operations. During field tests on the Athabasca River in June of 1974 and 1975, Flannagan et al. (1979, 1980) noted appreciable numbers of white sucker fry in drift nets downstream from the site of application of methoxychlor. While this could be the result of variables unrelated to larviciding, it could be attributable to a direct toxic action of the insecticide, to a methoxychlor-induced reduction in available food organisms, or to a combination of both.

This work was undertaken to test the null hypotheses that age, food availability, and preexposure of eggs to methoxychlor have no significant effect on the acute toxicity of methoxychlor to larval white sucker and that environmentally realistic pulse-exposure to methoxychlor will not significantly affect survival or wet weight gain. The research is a portion of a study undertaken to investigate the effects of pulse-exposure to methoxychlor on reproduction, as well as juvenile growth and survival, in fish. Previous work on American flagfish (*Jordanella floridae*) demonstrated impacts in terms of acute lethality (Holdway and Dixon 1985), effects on neurotransmitter levels (Holdway et al. 1986), effects of egg exposure on hatchability, larval serotonin levels and larval methoxychlor tolerance (Holdway and Dixon 1986a), and effects on growth and reproduction over one life cycle (Holdway and Dixon 1986b). The results presented here allow comparison of the responses of the flagfish, an exotic species used for detailed laboratory studies, with those of the white sucker, a more ecologically relevent indigenous species.

Materials and Methods

Adult white sucker were seined from the mouth of the 6.5°C Talbot River (Lake Simcoe, Ont.) on April 18, 1984. Eggs from two females were fertilized with milt from two males using wet fertilization methods. Cornstarch was added to dissociate the polysaccharide egg matrix. The eggs were transported to the laboratory at the University of Waterloo in 12 L of river water (arrival temperature 13.7°C) and distributed to 24 polyethylene strainers (diameter 12 cm; water depth 5 cm; effective water volume 0.5 L) which floated on the water surface in one of four 58-L holding tanks. Each tank was aerated and received 60 L wellwater·h^{-1} at 14.8°C.

The following morning the number of eggs in each test group was adjusted to 300. Starting at 14:00, four groups of eggs (designated as 1-d-old) were exposed for 2 h to a mean assayed methoxychlor concentration of 4.83 (SE 0.76) mg·L^{-1}. Subsequently, two groups each of 3- and 6-d-old eggs were similarly exposed. On completion of exposure the eggs were returned to toxicant-free water. Sixteen groups of untreated eggs were retained as controls.

During development the eggs were subjected daily to a 5-min dip in malachite green (4 mg·L^{-1}) to inhibit fungal growth. At the first sign of hatch (8 d postfertilization), antifungal treatment was discontinued. Since the eggs hatched over a 2-d period, percent hatch and incidence of gross deformities (criteria: ocular–cephalic, spinal, and greatly reduced size, McKim 1985) were determined 2 d posthatch. Onset of feeding was assessed 4 d posthatch.

At 2 d posthatch (10 d postfertilization), the temperature in the hatching tanks was increased to 20°C over a 14-h period. The larvae (designated as 1-d-old) were then transferred to 23-L glass aquaria for rearing. We used four aquaria for control larvae, two for larvae exposed to methoxychlor as 1-d-old eggs, and one each for larvae exposed as 3- and 6-d-old eggs. Each aquarium received a continuous flow of wellwater at a rate of 120 L·d^{-1}. Photoperiod was maintained at 16 h light : 8 h dark with 0.5 h of dawn and dust included in the light period. Larvae were fed to satiation five times daily, twice with live brine shrimp napulii and three times with a finely ground mixture of commercial diets (equal portions by weight of Floating Trout Feed Grower Pellets, Reg. No. 24320, Martin Feed Mills, Elmira, Ont., and Tetramin). The temperature in each aquarium was measured daily. Hardness, dissolved oxygen, alkalinity, and pH were measured weekly in randomly selected aquaria. Mean (SE) conditions were as follows: temperature 20.1 (0.24)°C; pH, 7.81 (0.07); dissolved oxygen, 9.5 (0.4) mg·L^{-1}; total hardness, 385.0 (19.0) mg·L^{-1} as $CaCO_3$; calcium hardness, 271.0 (8.4) mg·L^{-1} as $CaCO_3$; magnesium hardness, 114 (21.1) mg·L^{-1} as $CaCO_3$; alkalinity, 280.0 (5.5) mg·L^{-1} as $CaCO_3$.

Acute lethality tests were undertaken to determine the effects of age, food availability level, and embryonic exposure to methoxychlor on toxicity. The relative tolerance of subsamples of larvae was assessed by determining the 2-h pulse-exposure concentration of methoxychlor causing 50% mortality (PE LC50) during the subsequent 96–168 h. Bioassays were carried out on four groups of 2-d-old larvae (control and preexposed as 1- 3-, or 6-d-old eggs), four groups of 10-d-old larvae (control: fed and starved; preexposed as 1-d-old eggs: fed and starved), two groups of 18-d-old larvae (control: fed and starved), and two groups of 26-d-old larvae (control: fed and starved). All bioassays were carried in duplicate for a total of 24 tests.

Each bioassay exposed groups of larvae (10 per group) to mean (SE) assayed methoxychlor concentrations of 0.01 (—), 0.10 (0.02), 0.75 (0.13), or 2.92 (0.18) mg·L^{-1} for 2 h. Control and ethanol-carrier control groups were also undertaken. The carrier control group was exposed to 175 mg ethanol·L^{-1}, the maximum concentration of carrier used for any treatment. Bioassay chambers consisted of 100-mL linear polyethylene beakers modified by replacing the bottom with nylon screen and adding an exterior styrofoam flotation ring. Both before and after exposure, the six chambers that constituted one bioassay set were held in 23-L glass aquaria which received 120 L toxicant-free water·d^{-1}.

Methoxychlor (98% pure; 1,1,1-trichlor-2,2-bis(*p*-methoxyphenol)ethane, anhydrous MW = 345.6; Sigma Chemical Co.) was added to 23-L toxicant exposure tanks in a 95% ethanol carrier from a 16 g·L^{-1} stock 15 min prior to the introduction of larvae. Exposure tanks were vigorously mixed and aerated to ensure even toxicant distribution. To start a bioassay, the bioassay chambers containing the white sucker larvae were transferred to the exposure tanks for a 2-h period. The entire volume of each chamber was manually exchanged by raising and lowering the chambers every 15 min. Water samples for

TABLE 1. Mean (SE) hatching success and incidence of deformities in 2-d-old white sucker larvae exposed to methoxychlor for 2 h as eggs.

Methoxychlor exposure concentration ($mg \cdot L^{-1}$)	Age of eggs at exposure (d)	No. of replicates[a]	Hatchability (%)	Incidence of deformities (%)
0	—	16	65.5 (0.63)	1.1 (0.16)
4.83	1	4	66.4 (0.77)	2.9 (0.42)[c]
4.83	3, 6[b]	4	68.4 (1.72)	3.1 (0.71)[c]

[a]Each replicate contained 300 eggs.
[b]Results for days 3 and 6 were pooled for analysis.
[c]Significantly different ($p \leq 0.05$).

each bioassay were collected from each exposure tank 1 h after the start of exposure; 5-mL aliquots were pipetted from within the chambers and transferred into 15-mL glass vials to which 0.5 mL of *n*-hexane was added. Each vial was sealed, rapidly shaken, and stored at 4°C for analysis. At the end of the exposure the chambers were returned to the clean-water holding tanks after a rapid rinse in the ethanol-carrier control tank to remove any methoxychlor precipitate on the chambers (Holdway and Dixon 1985). The incidence of mortality was recorded 1, 2, 4, 8, 16, 24, 48, 72, and 96 h after exposure. Except where the design required that the fish not be fed, holding and water conditions did not differ significantly from those for larval rearing given above.

Water samples were analyzed for methoxychlor concentrations by electron-capture gas chromatography at the Pesticide Residue Laboratory, Ontario Ministry of Agriculture and Food, University of Guelph, Guelph, Ont. Analysis of variance showed that there were no significant differences in assayed concentrations between tanks having the same nominal concentration over all experiments. Therefore, all measurements were pooled and the means used as the best indicators of overall methoxychlor exposure.

Procedures used in the 30-d growth experiment were similar to those used in the lethality study. Twenty-four groups of 9-d-old larvae (30 per group) were placed in 12-cm polyethylene strainers. Four strainers were suspended at the top of each of six 23-L aquaria. Larvae from control eggs were placed in 12 of the strainers, while larvae from 1-d-old preexposed eggs were placed in the remaining 12 strainers. The larvae were exposed for 2 h to mean (SE) assayed methoxychlor concentrations of 0.10 (0.02), 0.75 (0.13), 2.92 (0.18), and 5.40 (0.56) $mg \cdot L^{-1}$, plus control and ethanol-carrier control conditions. All treatments were duplicated. Holding conditions did not differ significantly from those for larval rearing given above. Mortality was assessed daily. After 30 d, fish from the control, carrier control, and 0.10 mg methoxychlor $\cdot L^{-1}$ tanks (total mortality occurred at all higher concentrations) were anaesthetized with MS222 and length and wet weight were determined.

Data were analyzed using standard SAS programs from the library of the Department of Computer Services, University of Waterloo. The results for each bioassay were subjected to probit analysis (Finney 1971) to determine the median lethal concentration, the slope of the probit line, and 95% fiducial limits. If no significant difference occurred between replicates, data were pooled. The standard error of the difference (Sprague and Fogels 1977) was used to determine significant differences between bioassays. Data on hatchability, incidence of deformities, length, and weight were subjected to two- and three-way analysis of variance. Duncan's multiple range test was used to distinguish differences between means ($p \leq 0.05$).

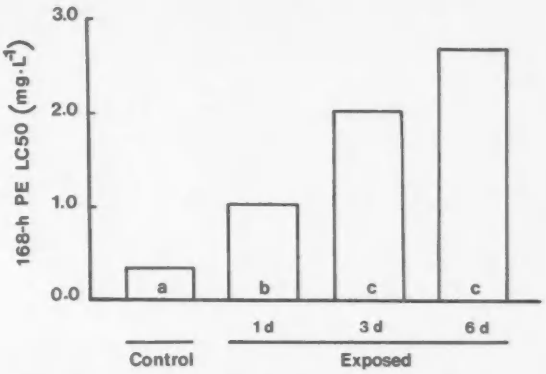

FIG. 1. Effect of methoxychlor preexposure of white sucker eggs of varying age on the subsequent acute toxicity of methoxychlor to the resulting 2-d-old larvae. Histogram columns without a letter in common are significantly different ($p \leq 0.05$).

Results

Exposure of white sucker eggs to methoxychlor 1, 3, or 6 d postfertilization did not alter hatchability; hatching success approximated 67% regardless of treatment (Table 1). Methoxychlor exposure did increase the incidence of gross deformities (Table 1). Eggs exposed at 1 d postfertilization showed 2.9% deformities, while those exposed at 3 or 6 d showed 3.1% These incidences were both significantly elevated relative to the 1.1% rate shown by controls.

Methoxychlor exposure also delayed the onset of exogenous feeding in white sucker larvae. While 96% of control larvae were feeding exogenously by 4 d posthatch, none of the larvae from the exposed groups had commenced feeding. By 5 d posthatch, all control larvae were feeding, while only 35, 37, and 57% of the larvae exposed to methoxychlor as, respectively, 1-, 3- or 6-d-old eggs were feeding. All larvae were feeding by 6 d posthatch.

Preexposure of white sucker to methoxychlor as eggs significantly altered the subsequent acute toxicity of methoxychlor to 2-d-old larvae, providing a tolerance that increased with increasing age at preexposure (Fig. 1). Larvae hatched from 1-d-old preexposed eggs showed a 168-h methoxychlor PE LC50 (slope of probit line, 95% fiducial limits) of 1.04 $mg \cdot L^{-1}$ (1.55, 0.83−1.32 $mg \cdot L^{-1}$), significantly higher than the control 168-h PE LC50 of 0.36 $mg \cdot L^{-1}$ (0.60, 0.21−0.63 $mg \cdot L^{-1}$). Similarly, 2-d-old larvae hatched from eggs preexposed at 3 and 6 d postfertilization showed respective 168-h PE LC50s of 2.05 $mg \cdot L^{-1}$ (1.33, 1.50−1.80 $mg \cdot L^{-1}$) and 2.67 $mg \cdot L^{-1}$ (1.10, 1.77−4.05 $mg \cdot L^{-1}$), significantly more tolerant of methoxychlor than either control larvae or larvae hatched from eggs preexposed as 1-d-old eggs.

The bioassays with 2-d-old larvae were carried out during the period of development when inflation of the swimbladder normally occurs. During the bioassays we observed that, regardless of the occurrence of mortality, all 2-d-old larvae hatched from control eggs and exposed to methoxychlor concentrations ≥ 0.10 $mg \cdot L^{-1}$ failed to inflate their swimbladders. Larvae of the same age hatched from 1-, 3-, and 6-d-old preexposed eggs all inflated their swimbladders at methoxychlor concentrations

TABLE 2. Summary of experimental protocol and pulse-exposure LC50s for white sucker larvae. Details of design, slopes of the probit lines, and 95% fiducial limits are given in the text.

Age at pulse exposure (d)	Food availability during bioassay	Length of bioassay (h)	PE LC50s ($mg \cdot L^{-1}$) for larvae hatched from			
			control eggs	eggs preexposed at 1 d	eggs preexposed at 3 d	eggs preexposed at 6 d
2	Unfed	168	0.36	1.04	2.05	2.67
10	Fed	96	0.04	0.18	—	—
	Unfed	96	0.05	0.09	—	—
18	Fed	96	0.34	—	—	—
	Unfed	96	0.03	—	—	—
26	Fed	96	0.20	—	—	—
	Unfed	96	0.04	—	—	—

TABLE 3. Impact of methoxychlor on total length and wet weight of 39-d-old white sucker larvae. The fish were exposed to methoxychlor either once as 1-d-old eggs (Group 1) or twice as 1-d-old eggs and 9-d-old larvae (Group 2). Unequal sample size necessitated separate analysis for each group. Within columns for each group, values not followed by the same letter are significantly different.

Methoxychlor concentration, egg exposure ($mg \cdot L^{-1}$)	Methoxychlor concentration, larval exposure ($mg \cdot L^{-1}$)	Sample size	Mean (SE) total length (mm)	Mean (SE) wet weight (mg)
Group 1				
0	0	57	21.8 (0.34)a	61.5 (3.73)a
0	0[a]	49	21.1 (0.39)a	58.5 (3.74)a
Group 2				
4.83	0	29	23.3 (0.52)a	78.0 (6.61)a
4.83	0[a]	33	22.0 (0.47)a	76.7 (4.93)a
4.83	0.10	29	20.8 (0.87)b	62.4 (8.60)b

[a]Ethanol-carrier control.

≤ 0.10 $mg \cdot L^{-1}$, while inflation was completely impaired at concentrations ≥ 0.75 $mg \cdot L^{-1}$.

The trend to increased tolerance with preexposure at the 1-d-old egg stage was also evident in both fed and starved 10-d-old larvae (Table 2). Control fed and starved larvae showed respective 96-h methoxychlor PE LC50s of 0.04 $mg \cdot L^{-1}$ (0.72, 0.01–0.08 $mg \cdot L^{-1}$) and 0.05 $mg \cdot L^{-1}$ (1.42, 0.03–0.07 $mg \cdot L^{-1}$). Preexposed fed and starved 10-d-old larvae showed significantly higher respective 96-h PE LC50s of 0.18 $mg \cdot L^{-1}$ (1.55, 0.14–0.22 $mg \cdot L^{-1}$) and 0.09 $mg \cdot L^{-1}$ (1.86, 0.07–0.13 $mg \cdot L^{-1}$).

After the onset of exogenous feeding, food deprivation increased the acute toxicity of methoxychlor to white sucker larvae. This is evident in the data for 10-d-old larvae given above. The 96-h PE LC50 for preexposed fed larvae (0.18 $mg \cdot L^{-1}$) was significantly greater than the PE LC50 for starved preexposed larvae (0.09 $mg \cdot L^{-1}$). Although there were no significant differences between fed and starved 96-h PE LC50s of control 10-d-old larvae (0.05 and 0.04 $mg \cdot L^{-1}$, respectively), significant differences were evident between older fed and starved control larvae (Table 2). Fed 18-d-old control larvae showed a 96-h PE LC50 of 0.34 $mg \cdot L^{-1}$ (0.86, 0.25–0.45 $mg \cdot L^{-1}$),significantly greater than the starved 96-h PE LC50 of 0.032 $mg \cdot L^{-1}$ (2.57, 0.026–0.039 $mg \cdot L^{-1}$). Similarly, fed 26-d-old control larvae had a 96-h PE LC50 of 0.20 $mg \cdot L^{-1}$ (1.45, 0.17–0.23 $mg \cdot L^{-1}$, significantly higher than the starved 96-h PE LC50 of 0.038 $mg \cdot L^{-1}$ (2.25, 0.034–0.042 $mg \cdot L^{-1}$).

Where sufficent numbers of larvae survived to allow valid comparison, pulse-exposure to methoxychlor significantly altered the growth of 9-d-old larvae over the subsequent 30-d period (Table 3). For 9-d-old larvae (hatched from eggs that had not been preexposed to methoxychlor) exposed for 2 h to 0, 0 plus ethanol–carrier, 0.10, 0.75, 2.92, or 5.40 mg methoxychlor $\cdot L^{-1}$, the incidences of mortality during the subsequent 30-d period were, respectively, 5, 18, 75, 100, 100, and 100%. The high levels of mortality permitted valid statistical comparison of total length and wet weight for only the control and ethanol-carrier control groups, between which there were no significant differences. For 9-d-old larvae (hatched from eggs that had been subjected to a 2-h exposure to 4.81 $mg \cdot L^{-1}$ as 1-d-olds) exposed for 2 h to 0, 0 (ethanol-carrier control), 0.10, 0.75, 2.92, or 5.40 mg methoxychlor $\cdot L^{-1}$, the incidences of mortality were 52, 45, 52, 100, 100, and 100%,respectively. The size of the fish in the 0.10 mg methoxychlor $\cdot L^{-1}$ group was significantly reduced, relative to both control groups (Table 3).

Discussion

Exposure of white sucker eggs to 4.83 mg methoxychlor $\cdot L^{-1}$ had no significant effect on hatchability, but significantly increased both the percentage of gross deformities and the age at which exogenous feeding began. Since exposure only raised the incidence of deformaties from 1 to 3%, and since the exposure concentration was approximately an order of magnitude higher than that which would occur

during larviciding operations in western Canada, the biological significance of these findings is questionable.

Methoxychlor exposure of white sucker eggs significantly increased the subsequent methoxychlor tolerance of the resulting larvae. Regardless of age or feeding status, larvae hatched from exposed eggs were consistently more tolerant of methoxychlor, by factors ranging from 2.1 to 35.2, than those from control eggs. This is in direct opposition to flagfish (Holdway and Dixon 1986a) where methoxychlor exposure of eggs causes decreased tolerance in the resulting juveniles. The increased white sucker tolerance also appears to be the exception to the generalized hypothesis that while acclimation to naturally occurring chemical stressors (chemicals present in the environment of a species over the course of its evolutionary history) will lead to increased tolerance, acclimation to xenobiotics will not (J. B. Sprague, University of Guelph, Guelph, Ont. N1G 2W1, pers. comm.).

The finding that preexposure of white sucker embryos induces limited tolerance may be important in mitigating acute mortality during larviciding operations. Lockhart et al. (1977) found that flathead chub (*Platygobio gracilis*) had not started to spawn at the time of methoxychlor treatment of the Athabasca River and the ripening ovaries contained from 0.03 to 9.33 μg methoxychlor·g^{-1}. These methoxychlor burdens could potentially offer some degree of protection if the larvae were subsequently reexposed to methoxychlor. This is not inconceivable, since methoxychlor is often applied two or three times to the same river at 2-wk intervals (NRC 1983).

The protective effects of exposure at the egg stage were not limited to tolerance. Larvae from eggs exposed to methoxychlor 1 d after fertilization successfully developed their swimbladders after a 2-h exposure to 0.1 mg methoxychlor·L^{-1}, while swimbladder development was completely arrested in exposed larvae from control eggs. The failure of swimbladders to inflate in exposed larvae suggests that methoxychlor interfers with development. The interference may occur at the neuroendocrine level, since a 2-h exposure to methoxychlor reduced brain levels of the neuroamine serotonin in adult flagfish (Holdway et al. 1986) and whole-body levels of serotonin in juvenile flagfish (Holdway and Dixon 1986a). Serotonin has been implicated in the control of gas balance in the swimbladder of the goldfish (*Carassius auratus*) (Olivereau et al. 1980) and the eel (*Anguilla anguilla*) (Oliverau and Oliverau 1977). Inadequate inflation of the swimbladder would prevent buoyancy regulation and impair feeding on surface plankton, severely limiting the performance of fish in the natural environment.

The acute toxicity of a 2-h pulse-exposure to methoxychlor was modified both by age and feeding status of white suckers. Considering only larvae derived from control (nonexposed) eggs, 10-d-olds, with a PE LC50 of 0.04 mg·L^{-1}, were significantly less tolerant than 2-, 18-, and 26-d-old larvae, with respective PE LC50s of 0.36, 0.34, and 0.20 mg·L^{-1}. While the sensitivity at 10 d is not fully understood, it may be related to the transition from endogenous to exogenous feeding, which started at 6 d and was progressing at this point. This is partially supported by the modifying effect of ration level on toxicity. While there was no effect of food deprivation at 10 d, starved larvae were significantly less tolerant than fed larvae at 18 and 26 d. This difference in toxicity with ration is possibly due to the energetic costs of either the convulsive activity which accompanies methoxychlor exposure to both white sucker larvae and juvenile flagfish (Holdway and Dixon 1986a) or the metabolic costs of detoxification. These costs could also explain the reduction in growth during the 30-d period following exposure reported here.

White sucker larvae were considerably less tolerant of methoxychlor than would be expected based on our previous work with flagfish. The 96-h PE LC50 for 2- to 26-d-old white sucker ranged from 0.04 to 0.36 mg·L^{-1} ($\bar{Y}$ = 0.23 mg·L^{-1}). Similar PE LC50s for 2- to 8-d-old flagfish ranged from 3.2 to 38.6 mg·L^{-1} ($\bar{Y}$ = 18.4 mg·L^{-1} (Holdway and Dixon 1985). Comparing means, it is apparent that white sucker are approximately two orders of magnitude more sensitive to methoxychlor than flagfish. This interspecies difference is also evident, although to a lesser degree, for LC50s based on continuous exposure. Waiwood and Johansen (1974) reported a 96-h LC50 of 0.035 mg·L^{-1} for 20-g white sucker, 8.3 times lower than the 96-h continuous-exposure LC50 of 0.29 mg·L^{-1} that we reported for adult flagfish (Holdway and Dixon 1985).

A 2-h pulse-exposure to 0.25 mg methoxychlor·L^{-1} of 8-d-old flagfish results in both some degree of reproductive failure in those fish as adults and significant deformities in their offspring (Holdway and Dixon 1986b). A 2-h pulse-exposure to 0.10 mg methoxychlor·L^{-1} completely inhibited swimbladder development in 2-d-old white sucker and caused 50–75% mortality, as well as reduced growth, in 9-d-old white sucker after 30 d. Since this value is three times lower than 0.30 mg·L^{-1} concentration of methoxychlor used to control blackflies in the Athabasca and Saskatchewan River systems (NRC 1983), it is not unrealistic to expect a significant toxic response by larval white sucker to larviciding operations in these rivers.

Acknowledgments

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Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript Submission

Submit manuscripts in triplicate to the Editor, accompanied by a covering letter that (1) states the main points and significance of the work, (2) accepts the mandatory $44 page charge, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. The page charge can be waived only if the work is of unusually high quality and grant support is lacking. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

Editorial Practices

The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (4th ed. 1980), Spec. Publ. No. 12, American Fisheries Society. All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty reprints are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs).

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 21.5×28 cm (8.5 × 11 in.), on only one side of the page. If possible, use paper with lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units of measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as g $C/m^2/d$; use g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when galley proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against text citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints of figures, or originals if they do not exceed 21.5 × 28 cm, on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract

enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as "... is discussed" or "... was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exeed 375−500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine ... we ..." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Empahsis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit within either a column or page of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions or the original artwork (no larger than 21.5 × 28 cm) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures should be avoided if possible, as they usually reproduce poorly. Dot-matrix® printers cannot produce sufficiently dark, solid lines to ensure legibility. Figures drafted in black india ink are always preferable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetre per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	*M*
mole	mol
moles per litre	mol/L, *M*
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. comm."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)." Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

PETERMAN, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

GORDON, D. C. JR., AND A. S. HOURSTON [ED.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LEBLOND, P. H., AND L. A. MYSAK. 1978. Waves in the ocean. Elsevier, New York, NY. 602 p.

Part of book

HEALEY, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia, p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

KUTTY, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, Univ. Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

SMITH, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sous-jacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible ; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Notes : communications brèves signalant une connaissance nouvelle et significative découlant de problèmes étroitement limités et facilement définis, ou d'importantes découvertes qui méritent d'être publiées avant que des études plus vastes soient terminées. Les notes ne doivent pas dépasser 3 pages imprimées (9 pages de manuscrit).

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata, et accompagnés d'une lettre mentionnant : (1) les principaux points et l'importance du travail, (2) l'acceptation des frais de publication obligatoires de 44 $ par page, (3) des suggestions quant aux arbitres éventuels et (4) une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. Les frais de publication pourront ne pas être débités aux auteurs qui n'ont pas reçu de subventions et dont le travail est d'une qualité exceptionnelle. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103. C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (4e éd. 1980), Spec. Publ. No. 12, American Fisheries Society. Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, n° 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Cinquante tirés à part sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication (un bon de commande à cet affet accompagne les épreuves).

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les

articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 21,5 sur 28 cm (8,5 sur 11 pouces), sur un côté de la page. Si possible, employez un papier avec lignes numérotées en marge. Laissez toujours une marge d'au moins 2,5 cm.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun endroit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles ? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constatations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, data, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux et le texte, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que g $C/m^2/d$; utilisez plutôt g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que chaque tableau et figure sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les legendes doivent indiquer le but du tableau on de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Département de l'économique, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al*.

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demende, lorsqu'il s'agira de publier l'article, envoyez des photographies des figures, ou encore les originaux, s'ils ne dépassent pas 21,5 sur 28 cm. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés

généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : (1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et (2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à ... » ou « Recherches sur ... ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquex au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la comprehension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que « ... on y analyse » ou « ... a été trouvé » ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que est c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail.

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autre informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375−500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phase qui en indique le but, comme : «Afin de déterminer ... nous ...» Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possibles, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synethèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux at communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (les adresses incluant). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'encroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondre à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se

répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à une ou deux colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplementaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit « Tableau — près d'ici ».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies ou les dessins originaux (pourvu qu'ils ne dépassent pas 21,5 sur 28 cm) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, $1\frac{1}{2}$ ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig.—près, d'ici ».

On devra autant que possible éviter les illustrations préparées par ordinateur, qui ne se reproduisent pas très bien. Les imprimantes par points ne peuvent produire de lignes solides suffisamment foncées pour être lisibles. On obtiendra toujours de meilleurs résultats en utilisant de l'encre de Chine noire. Si vous utilisez du lettrase instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la letter *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètre par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	cm
degré Celsius	°C
degrés de liberté	dl
écart type	EcT
élecronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	*M*
mole	mol
moles par litre	mol/L, *M*
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour−mois−année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le

nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : « Brown (1983) compare . . . » ou « . . . on a comparé (Brown 1983) ». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « *et al.* » : « Brown *et al.* (1983) comparent . . . » ou « . . . on a comparé (Brown *et al.* 1983) ».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « comm. pers. »

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-les dans la liste des références, avec la mention « (Sous presse) ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mains indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibiographiques (d'aprés les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieu subarctique. Can. J. Fish. Aquat. Sci. 40: 1278–1292.

Numéro complet du journal

GORDON, D. C., JR. ET A. S. HOURSTON [ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40 : (Suppl. 1) : 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans, Service hydrographique du Canada, Ottawa (Ont.). 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1972. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.] La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION ET WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14e éd. Washington, DC. 1193 p.

Thèse

DELISLE, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Univ. Ottawa, Ottawa (Ont.). 161 p.

Rapports

DUTIL, J. D., M. FORTIN ET Y. VIGNEAULT. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. manus. can. halieut. aquat 1653 : 29 p.

Traduction

KOIKE, A. ET M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64 : 1–11. (Traduit du japonais par Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles « h », « min » et « s » ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12:30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, « cold water » (n.) « cold-water » (adj.) ; « hard water » (n.), « hardwater » (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

Pascho, R. J., and D. Mulcahy. **Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease** 183–191
Davis, M. W., and B. L. Olla. **Aggression and variation in growth of chum salmon (*Oncorhynchus keta*) juveniles in seawater: effects of limited rations** 192–197
Vézina, A. F., and T. Platt. **Small-scale variability of new production and particulate fluxes in the ocean** 198–205

Notes

Elliott, D. G., and T. Y. Barila. **Membrane filtration – fluorescent antibody staining procedure for detecting and quantifying *Renibacterium salmoninarum* in coelomic fluid of chinook salmon (*Oncorhynchus tshawytscha*)** 206–210
Kostecki, P. T., P. Clifford, S. P. Gloss, and J. C. Carlisle. **Scale loss and survival in smolts of Atlantic salmon (*Salmo salar*) after turbine passage** 210–214
McDowell, W. H., J. J. Cole, and C. T. Driscoll. **Simplified version of the ampoule–persulfate method for determination of dissolved organic carbon** 214–218
Hardy, R. W., E. Casillas, and T. Masumoto. **Determination of vitamin B_6 deficiency in rainbow trout (*Salmo gairdneri*) by liver enzyme assay and HPLC analysis** 219–222
Pepin, P. **Influence of alternative prey abundance on pelagic fish predation of larval fish: a model** 222–227
Holdway, D. A., E. J. Kempe, and D. G. Dixon. **Acute toxicity of methoxychlor to larval white sucker (*Catostomus commersoni*) as modified by age, food availability, and egg preexposure** 227–232

Coming next month / À paraître le mois prochain 233–234
Guide for authors / Guide des auteurs i–x

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 44, No. 1, January 1987 **Volume 44, nº 1, janvier 1987**

Reinhart, J. M. **Editorial**

Articles

Johnson, M. G. **Trace element loadings to sediments of fourteen Ontario lakes and correlations with concentrations in fish** 3–13

Buckley, L. J., and R. G. Lough. **Recent growth, biochemical composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank** 14–25

Koslow, J. A., K. R. Thompson, and W. Silvert. **Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate** 26–39

Hayes, J. W. **Competition for spawning space between brown (*Salmo trutta*) and rainbow trout (*S. gairdneri*) in a lake inlet tributary, New Zealand** 40–47

Peterman, R. M., and M. J. Bradford. **Density-dependent growth of age 1 English sole (*Parophrys vetulus*) in Oregon and Washington coastal waters** 48–53

de Lafontaine, Y., and W. C. Leggett. **Evaluation of in situ enclosures for larval fish studies** 54–65

Blackett, R. F. **Development and performance of an Alaska steeppass fishway for sockeye salmon (*Oncorhynchus nerka*)** 66–76

Valett, H. M., and J. A. Stanford. **Food quality and hydropsychid caddisfly density in a lake outlet stream in Glacier National Park, Montana, USA** 77–82

Sanders, J. G., S. J. Cibik, C. F. D'Elia, and W. R. Boynton. **Nutrient enrichment studies in a coastal plain estuary: changes in phytoplankton species composition** 83–90

Uthe, J. F., and C. L. Chou. **Cadmium in sea scallop (*Placopecten magellanicus*) tissues from clean and contaminated areas** 91–98

Laurén, D. J., and D. G. McDonald. **Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology** 99–104

Laurén, D. J., and D. G. McDonald. **Acclimation to copper by rainbow trout, *Salmo gairdneri*: biochemistry** 105–111

Ho, K. T. Y., and S. P. Gloss. **Distribution and persistence of the lampricide Bayer 73 following a control application** 112–119

Morantz, D. L., R. K. Sweeney, C. S. Shirvell, and D. A. Longard. **Selection of microhabitat in summer by juvenile Atlantic salmon (*Salmo salar*)** 120–129

Axler, R. P., and J. E. Reuter. **A simple method for estimating the ^{15}N content of dissolved organic matter ($DO^{15}N$) in N-cycling studies** 130–133

Cochran, R. C. **Effects of coal leachates on fish spermatogenesis** 134–139

Donard, O. F. X., F. T. Short, and J. H. Weber. **Regulation of tin and methyltin compounds by the green alga *Enteromorpha* under simulated estuarine conditions** 140–145

Cornett, R. J., and F. H. Rigler. **Decomposition of seston in the hypolimnion** 146–151

MacDonald, B. A., and N. F. Bourne. **Growth, reproductive output, and energy partitioning in weathervane scallops, *Patinopecten caurinus*, from British Columbia** 152–160

Maule, A. G., C. B. Schreck, and S. L. Kaattari. **Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-to-smolt transformation and after implantation of cortisol** 161–166

Couture, P., C. Thellen, P. A. Thompson, and J.-C. Auclair. **Structure and function of phytoplanktonic and microbial communities in relation to industrial wastewater discharge: an ecotoxicological approach in a lotic system** 167–175

McCauley, E., and J. Kalff. **Effect of changes in zooplankton on orthophosphate dynamics of natural phytoplankton communities** 176–182

(continued inside back)

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